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423. SOME OBSERVATIONS ON THE BEHAVIOUR OF DAIRY CATTLE WITH PARTICULAR REFERENCE TO GRAZING

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(With 2 Plates and 4 Text-figures)

INTRODUCTION

Improvements in strains of grasses and clovers, increased knowledge of the correct use of fertilizers in larger quantities, and a general appreciation of the value of leys as a source of animal food have all helped to place at the dairy farmer's disposal highly productive leys. There is, however, little reliable information available as to the precise way in which leys should be used by a dairy herd in order to make full use of the sward nutrients that can be produced by a system of intensive grassland management. It is well known that there is a great difference in the milk output per acre on different farms even in the same area and where the opportunities to produce a good sward may be similar.

One reason for the absence of precise information on the proper use of leys is no doubt the paucity of reliable information on the normal behaviour of cattle, particularly while grazing. A small number of farmers, e.g. those in certain areas famed for their grazing pastures, have inherited the art of careful sward management, but a widespread improvement in grazing technique will require numerical presentation of this 'art'. Before this can be done accurately detailed studies of animal behaviour would appear to be essential. The need for this work has been emphasized by Edwards (1), Stapledon (2, 3) and others. The practical outcome of studies of this type has been illustrated by Nichols (4) for sheep and Stoddart & Rasmussen (5) for deer and range livestock.

The present study was commenced, first, to obtain a fairly detailed picture of the behaviour of dairy cattle with particular reference to grazing habits, and secondly, to use the data so obtained to make tentative suggestions for the improvement of grazing management.

REVIEW

Most of the studies of the behaviour of cattle that have been made concern specific problems of dairy herd and grassland management, and few include records of all the major activities of cattle throughout substantial periods of time. Much of the recorded quantitative information has, in fact, been incidental to investigations of other matters.

One of the first recorded studies was made by Cory (6) in order to obtain detailed information concerning the behaviour of cattle under range conditions in Texas. Thirty-six observations covering a period of 3 years showed that in a 'day' of 13·6 hr. (i.e. from getting up in the morning to bedding down at night), 461·4 min. was spent in feeding. Shepperd (7) observed the behaviour of three Hereford steers for a period of 24 hr. while grazing sweet clover and recorded the actions of the animals at 15 min. intervals. Grazing time occupied 495 min., mainly between 7.0 and 10.0 p.m., and 5.0 and 10.0 a.m. The main

period of lying down was from 10.0 p.m. to 5.0 a.m. The intensity of grazing was found by Giöbel & Lindbom (8) to be as great in the night as during the day, and alternated at fairly regular intervals with periods of rest. The animals spent approximately 50% of their time on pasture in effective grazing. This distribution of grazing is in close agreement with the results given by Johnstone-Wallace & Kennedy (9), who recorded the activities of beef cows at pasture. In 24 hr., 452 min. were spent grazing, 60% of this being during the day and 40% at night. Only 5 hr. of the grazing time was spent in actual eating; the remainder of the time was used in searching for the food. Anon (10), reporting the behaviour of five grazing bullocks, found that 93% of the 523 min. spent grazing during a 24 hr. observation occurred in the 'light' period (17 hr.). The main concentration of grazing was during the early hours of the morning, and again between late afternoon and the onset of darkness. Observations made at the same Station (11) in 1948 showed that 94% of the total grazing time occurred during daylight in April, and 98, 96 and 81% respectively in June, July and August. Daily grazing time varied from 410 to 491 min. Under New Zealand grazing conditions Hancock & Wallace (12) studied the behaviour of six sets of identical twin heifers over six 24 hr. periods at monthly intervals covering the period of lactation. During each 24 hr. period 403 min. were spent grazing, 58% occurring between 7.0 a.m. and 3.0 p.m.

The observation of animal behaviour has been used by some investigators as an integral part of studies in relation to pasture management. Cory (6) collected data under non-experimental conditions in order to develop a system of grazing that would utilize the range vegetation in the best way. Hein (13) recorded and compared the behaviour of grazing steers when subjected to different systems of management on pasture. Six 24 hr. observations covering two years were made to compare the animals' behaviour when grazing plots stocked at a rate of one animal per 2 acres, one animal per acre, and alternate plots at one animal per $\frac{1}{2}$ acre. As the quantity of herbage available to each of the groups was not kept constant, results were often conflicting, although, in general, grazing time was influenced more by the comparative abundance of the pasture than the individuality of the animal. Hein suggested that approximately $8\frac{3}{4}$ hr. per day were spent grazing if the herbage was plentiful and approximately 10 hr. if the herbage was short. About 66% of the grazing occurred during the daylight period from 6.0 a.m. to 6.0 p.m.

To compare the behaviour of four cows continuously grazing a pasture of 2 acres with that of four cows grazing in rotation six paddocks of $\frac{1}{3}$ acre each, Hodgson (14) recorded the length and number of times spent grazing. Observations were made from 6.0 a.m. to dark. The animals on the rotationally grazed plots grazed an average of 8·83 times and spent 411 min. grazing compared with the 7·87 times and 439 min. of the animals on the continuously grazed plots. As the season advanced the cattle which were rotationally grazed spent less time grazing and a greater time lying down, while the continuously grazed animals reacted in the reverse manner. Data given by Atkeson (15) would appear to confirm the above results, for on good pasture the cattle grazed an average of 5·6 hr., on fair pasture 6·5 hr., and on poor pasture 7·3 hr. during a 'day' of 11 hr. 40 min. These results are not confirmed by Johnstone-Wallace (16), who observed that cattle showed no inclination to graze longer than 8 hr. per day even when the amount of green herbage consumed fell from 150 to 45 lb. per day. Hancock (17) suggested that there is probably a correlation between grazing time and the individual's intake of dry matter, while Johnstone-Wallace & Kennedy (9) have related the weight of herbage per acre available in

a pasture to the weight of herbage consumed in 24 hr. If a relationship exists between the intake of dry matter and grazing behaviour, then the feeding of supplementary food to cattle at pasture should affect the time spent grazing. This was found by Moseley (18), who recorded the grazing time of animals on pasture alone as 9·16 hr., on pasture supplemented with alfalfa hay as 6·51 hr., and when a limited or full concentrate ration was fed as 5·60 and 5·33 hr. respectively. These observations were in a 'day' from 5.0 a.m. to 9.0 p.m.

Hancock (17) recorded that animals fed 1 lb. of concentrates per 5 lb. of milk produced, grazed for a shorter time per day than animals on pasture alone and that cattle were prepared to work for much longer hours if the pasture were short but still palatable. Seath & Miller (19) found no reduction in the time spent grazing when 4·7 lb. of hay were fed to cattle at pasture, although animals receiving 5·5 lb. per head per day grazed an average of 40 min. per day less than during the control period.

The effect of weather and, in particular, high temperatures upon the behaviour pattern of cattle has been noted. Bonsma (20), working in South Africa, observed animals of four different breeds during a 'day' from 8.0 a.m. to 5.0 p.m. A maximum temperature of 103° F. occurred at 3.0 p.m. Afrikaner animals spent 89% of the day grazing compared with 79% for Herefords, 78% for Shorthorns and 75% for Aberdeen Angus. Under American conditions Seath & Miller (19) observed that Holstein cattle grazed on average 1·49 hr. in the sunshine and 0·4 hr. in the shade during the day, whilst 4·7 hr. were spent grazing at night. When air temperatures were at the lowest (81° F. at 3.0 p.m.) grazing occupied 2·43 hr. in the daytime compared with 1·14 hr. when the air temperature at 3.0 p.m. was 91° F. This was in agreement with earlier results (Seath & Miller (21)), when it was observed that cattle grazed three times as much at night than during the day on warm days, and 2·4 times as much on cool days. Hancock & Wallace (12) found that hot weather (77° F.) depressed overall grazing time, but did not alter the day and night grazing ratio.

By the observation of the animals' behaviour it has been possible to aid the study of specific problems of dairy-herd management. When investigating feed flavour in butter in New Zealand, Bruce-Levy (22) made herd observations. Little grazing was observed between midnight and the morning milking, and he suggested that this may be the cause of less feed flavour in the morning's than in the evening's cream.

EXPERIMENTAL

The experimental work was divided into two parts: (*a*) the detailed observation of the behaviour of four cows for periods of 24 hr., and (*b*) the observation of the behaviour of a dairy herd of approximately fifty animals at monthly intervals for a year.

(*a*) *Observations on four cows*

The purpose of these observations was to record, over periods of 24 hr., the time which individual cows spent in grazing, lying down, loafing and ruminating, and the distance walked. The number of times the animals drank, defaecated and urinated was also recorded.

Method

Behaviour categories were defined as follows:

Grazing time was the entire time the cow spent searching for and eating herbage.

Lying-down time was the total time the animals were reclining whether ruminating or not.

Loafing time was the difference between the sum of the two previous times and 24 hr., i.e. the time spent walking and standing up but doing nothing specific except some chewing of the cud.

Ruminating time was the entire period covered by the acts of regurgitation, the chewing of the bolus, and deglutition, including intervals between boluses, whether the animal was standing up or lying down.

Each observation commenced at 8.0 a.m. and continued for 24 hr. Four mature Dairy Shorthorn cows in milk were driven into a 1-acre square paddock temporarily constructed using electric fencing equipment as described by Castle & Foot (23). The paddock contained a large tree for shade, with a water trough along one side. Two or three observers, working on a shift system, observed the cattle continuously until 8.0 a.m. the following day, milking times included. Records were kept on specially printed sheets. Times were recorded, to the nearest minute, at which each cow changed her behaviour, e.g. commenced grazing after a period of loafing, and also when dung and urine were voided and when drinks were taken.

During the hours of darkness it was found quite easy to record the behaviour of the animals by fairly close observation, once the eyes of the observers were adjusted to the darkness, and by listening keenly for any movement. A dimmed spotlight beam from a flash lamp was used to only a very limited extent.

The method of recording the distance a cow walked presented difficulties, and it was only possible to obtain this information for one of the four cows. To do this, the paddock was divided into sixteen equal squares by driving posts into the ground, so that by judging the position of the animal in relation to a post it was possible to draw upon a scale plan of the paddock the track which one of the cows followed. The length of this track was measured on the plan by a cyclometer and converted by the appropriate scale to give the distance which the cow walked. 88% of all observations and records were made by two of the authors and when the recording technique had been tested, and both observers interpreted the behaviour of the same animal in an identical way, four 24 hr. observations were made. Some details of these are given in Table 1.

Table 1. *Details of four-cow observations*

Observation no.	Date	Type of grazing	Temperature (° F.)		Weather		
			Max.	Min.	Rain	Wind	General conditions
1	26–27 Aug. 1948	Old ley*	63	45	Nil	S.W.	Clear sunny day, fine moonlight night
2	2–3 Sept. 1948	Young ley	63	53	30 min. shower in evening	S.W.	Dull, overcast day, clear fine night
3	9–10 Sept. 1948	Old ley*	76	57	Nil	S.–S.E.	Clear sunny day, fine bright night
4	21–22 Oct. 1948	Old ley	62	54·5	12 min. shower in morning	S.W.	Dull day, cloudy night

* Same ley.

Results

The results of the four observations and the weight of milk produced by each animal during the observed period are presented in Table 2.

Table 2. *Results of four-cow observations*

Observation no. ...	1				2				3				4					As % of 24 hr.
Animal	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	E*	Mean	
Time spent (hr.):																		
Grazing	7·60	5·68	10·48	8·08	6·55	5·57	5·30	6·07	6·30	7·37	7·50	8·07	4·33	5·05	4·97	5·40	6·52	27·2
Lying down	7·73	7·85	6·10	9·18	9·25	9·22	9·12	9·12	9·12	8·77	8·63	9·17	10·83	11·48	11·32	10·65	9·22	38·4
Loafing	8·67	10·47	7·42	6·74	8·20	9·21	9·58	8·81	8·58	7·86	7·87	6·76	8·84	7·47	7·71	7·95	8·26	34·4
Ruminating (lying down and loafing)	5·55	4·75	3·72	4·70	6·65	5·97	6·75	5·97	5·45	4·80	4·65	4·77	6·48	6·63	6·43	6·32	5·60	—
Number of:																		
Defaecations	10	9	10	12	11	12	13	15	10	12	13	13	7	11	14	13	11·6	—
Urinations	9	12	10	9	9	12	14	9	7	15	12	10	5	8	9	7	9·8	—
Drinks	3	5	4	4	3	4	2	2	5	2	5	7	2	4	6	2	3·8	—
Milk yield (lb.)	36·25	35·75	46·00	28·50	36·00	33·50	54·25	32·00	36·25	32·25	52·25	31·25	28·00	31·75	41·50	28·75	—	—
% of grazing time between a.m. and p.m. milkings	44	43	42	58	38	43	45	41	54	63	52	56	46	50	48	55	49	—
Distance walked (yd.)	3960	—	—	—	1950	—	—	—	3240	—	—	—	3090	—	—	—	3060	—

* Cow D replaced by cow E in observation no. 4.

Grazing time. The average time spent grazing during the observations was 6·52 hr. per day, and ranged from 4·33 to 10·48 hr. During observation no. 1 when the cows were on an old ley they grazed for a longer period than during the second observation when they were on a new ley with 6–8 in. of leafy growth. When the same cows returned to the original old ley the time of grazing of three of the cows increased. The mean grazing time for the four cows during observations 1, 2 and 3 were respectively 7·97, 5·87 and 7·31 hr.

In observation 4, cow D was replaced by cow E, so the results are not strictly comparable with former ones, although a noticeable decline in grazing time for the three cows, common to all observations, is observed. The grazing available to the cows in this last observation was of a poor type from an old sward, and the herbage at this late season of the year may not have been as palatable as it was previously.

The proportion of the grazing time between a.m. and p.m. milkings did not vary widely either between cows or between pastures, approximately 50% of total grazing time being in this period. It was thought that there might be some relationship between grazing time and milk yield, but the number of separate observations was small and the correlations obtained were all non-significant. Minimum temperature exerted no obvious influence upon grazing time, but it was recorded that during warm sunny periods the cows grazed mainly in the shade.

Time spent lying down. The mean time spent lying down during the four observations was 9·22 hr., times for individual cows ranging from 11·48 to 6·10 hr. These times were not related to either the current daily milk yield of the animal or the temperature, but a correlation of −0·942 was found between the mean times spent lying down and the mean grazing time recorded in the four observations. In considering this figure it must be remembered that one of the original cows had been replaced, but it would appear that the longer the time a cow grazes, the less time she spends lying down. Only 14·5% of the total time spent lying down was between the a.m. and the p.m. milkings.

Time spent loafing. In our observations, loafing time had no particular significance in itself. It occupied 8·26 hr. of the cows' day (mean figure for all observations), but although approximately 3½ hr. of this was enforced because of a man-made milking routine, there is still a difference of over 4 hr. which was accounted for in the field.

Time spent ruminating. The average time spent ruminating per day was 5·60 hr., of which 4·52 hr. (81%) occurred whilst the cows were lying down. To illustrate the periodicity of rumination, Text-fig. 1 has been constructed. In this diagram the width of the black bar indicates the length of each ruminating period; above the line when the cow was loafing (i.e. on her feet) and below when lying down. The average length of a period of rumination was 35 min. It can be seen that the regularity of rumination was interrupted by periods of feeding both before and after the p.m. milking. In the four observations, eleven periods of rumination were recorded during the p.m. milking period but only two (the same cow at the same milking) in the a.m. milking. Throughout the observations it was noted that the cows preferred to ruminate whilst lying down; the exception to this was on the evening of observation 2 when, because of a heavy shower, the animals ruminated whilst loafing.

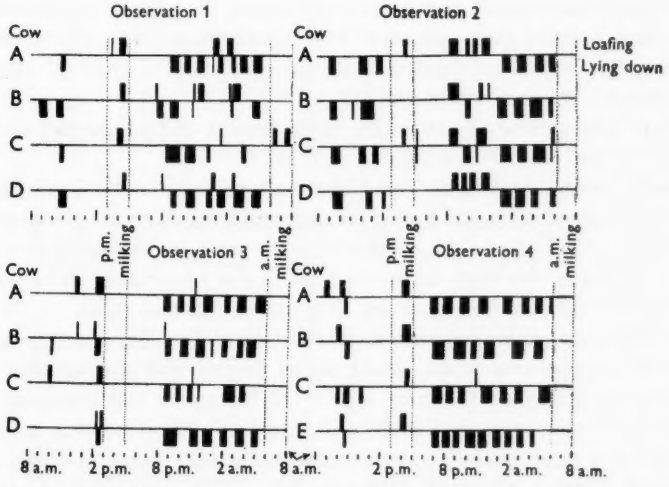
Defaecations and urinations. Dung was voided an average of 11·6 times, and urine 9·8 times in the 24 hr. period. The distribution of the dung and urine during this time is of interest, and further details are given in Table 3.

Number of drinks. The average number of drinks taken in 24 hr. was 3·8. It is worthy

of note that in observation 2 when the cattle grazed a lush young ley, the number of drinks per cow averaged 2·75, whilst the averages for observations 1 and 3, when the cattle were on an old and much barer ley, were respectively 4·00 and 4·75. 66% of the total number of drinks in 24 hr. were taken between the a.m. and p.m. milkings and 34% between the p.m. and a.m. milkings; none were taken from the waterbowls whilst the cattle were being milked. All the drinks between the p.m. and a.m. milkings were taken immediately after the cows were turned into the field.

Table 3. *Distribution of defaecations and urinations during the 24 hr. in the four-cow observations*

Period	% of total number	
	Defaecations	Urinations
During a.m. milking and on the road	10	5
Between a.m. and p.m. milkings (day pasture)	36	35
During p.m. milking and on the road	2	1
Between p.m. and a.m. milkings (night pasture)	52	59
	100	100



Text-fig. 1. Periods of rumination—four cows—four observations. Above line when loafing, below when lying down.

Distance walked. The distance walked in the paddock by the cow averaged 3060 yd. (1·74 mile), excluding a distance of 740 yd. to the cowshed which was travelled 4 times a day. It can be seen from Table 2 that when the cow was on the sparser herbage (observations 1, 3 and 4) she walked distances of over 3000 yd., whereas on the one occasion that she was on a ley supplying plenty of 'keep' she walked less than 2000 yd. 60% of the total distance travelled during the day was covered whilst grazing, and 40% whilst loafing. The average distance walked between a.m. and p.m. milkings was 59% of the total distance travelled in the 24 hr.

(*b*) *Herd observations*

In order to build up a picture of the behaviour pattern of a dairy herd under normal conditions of management, a series of herd observations was undertaken in the period

November 1948 to October 1949. Much of the data which were collected could not be interpreted in an exact numerical fashion, but from a general study of the behaviour pattern much valuable information has been gleaned.

Method

As in the four-cow observations the cow's day was divided into the time spent grazing (or eating), lying down and loafing, but detailed records were not kept of defaecations, urinations, or drinks. For these observations, a record of the number of animals eating, lying down, or loafing was made every 5 min. during the period of light and every 15 min. during the hours of darkness. Additional data were collected concerning the food and grazing available, prevailing weather conditions, and other relevant information. Each observation commenced at 8.0 a.m. and continued for 24 hr, milking times excluded. Herd numbers averaged fifty-two and consisted of Shorthorns with a few Guernseys, and, in the later observations, a few Friesians.

Results

Twelve herd observations were carried out during the year at approximately monthly intervals, and covered every phase of herd management. The results can be broadly divided into the behaviour pattern under (*a*) conditions of 'winter' management and (*b*) conditions of 'summer' management.

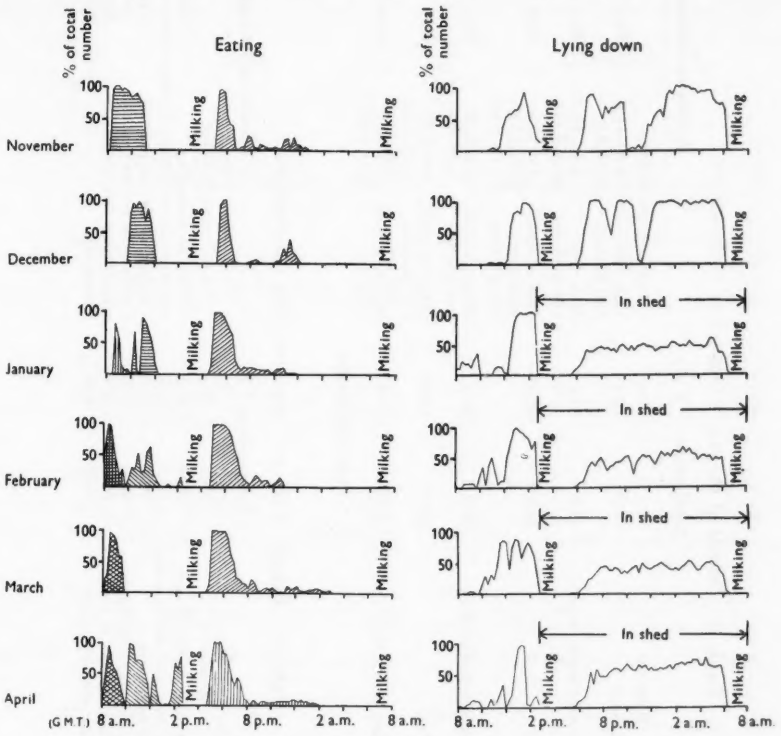
(*a*) *Winter management.* Some details of the feeding and management of the herd and prevailing weather conditions are given in Table 4.

The results of the six 'winter' herd observations are presented graphically in Text-fig. 2. Only details of eating (including grazing) and lying down are given, and to facilitate interpretation of the graphs the number of cows has been expressed as a percentage of the total number in the herd during each particular observation. The number of cows observed varied between forty-four and fifty-five on different dates.

Eating. The most striking feature in this section of Text-fig. 2 is the regularity of the shape of the graphs even when conditions of feeding and management are different. When these conditions are the same, e.g. in the November and December observations, the similarity is even more noticeable, and would appear to reflect a definite behaviour pattern under a given set of environmental conditions. Some of these conditions are fixed because of a time-governed milking routine, and it is apparent that there is a major peak of feeding immediately the animals enter the field after each milking or when the cattle have access to another food, e.g. during the January observation there is a second peak of feeding when the herd is turned on to the kale fold after a feed of hay in the cowshed. During the November and December observations, the herd in the field was observed to be very restless about midnight. The cows searched for and consumed remnants of hay and kale before lying down again until the a.m. milking. Later in the winter when in the cowshed at night odd animals picked at their food residues until the early hours of the morning, but no definite secondary peak of feeding was recorded similar to the one in the field. During the November and December observations, when hay and kale were fed simultaneously in the field, no preference was shown for either food, which was surprising in view of the fact that the animals had been on a kale fold in the morning. Concentrates were fed at each milking, but this has not been recorded on the graphs.

Lying down. The similarity of the lying-down curves of the herd when under the same

conditions of management is apparent from Text-fig. 2. During November and December, when the herd was out of doors all day and the behaviour of the herd was not restricted by any activity of man, very similar patterns were recorded. Three main periods of lying down occurred: (i) before the p.m. milking, (ii) between the evening feed and midnight, and (iii) between the period of activity around midnight and 6.0 a.m. During the night one or two animals always tended to loaf in preference to lying down, but in general the entire herd laid down in a sheltered position. From January to April the pattern of lying down before the p.m. milking was again very similar to the previous observations, but a definite change was noticeable in the period between the p.m. and a.m. milkings.



Text-fig. 2. The behaviour of a herd of dairy cows during the winter months.

Key to food eaten: grass, ▨; hay and roots, ▨; kale, ▨; silage, ▨; hay and silage, ▨; hay, ▨.

The animals were now housed at night and fastened in the normal type of tie-up standing (either yoke or chain ties) with adequate bedding. After the hay and mangolds had been eaten a tendency was shown for the cows to lie down, but this was not nearly so pronounced as at the comparable times when the herd was at pasture. Rarely did more than half the herd lie down at the same time; there was no period of activity around midnight, and in general the animals appeared very restless. At any one time during the night and early morning approximately 50% of the cows would be standing up, and judging from the constant rattle of chains and yokes throughout this period there was much movement and change of activity on the cows' part. This type of behaviour was

Table 4. *Details of the six 'winter' herd observations*

Observation no.	Date	Weather	Temperature (° F.) Max.	Min.	Feeding and management a.m.→p.m. milking		p.m.→a.m. milking
1	12–13 Nov. 1948	Fine and clear	54·0	45·0	Kale fold Old pasture	8.30–11.0 11.0–3.0	Hay and kale fed on old pasture
2	20–21 Dec. 1948	Fine and cold windy night	41·0	38·0	Kale fold Old pasture	9.45–12.0 12.0–2.45	Hay and kale fed on old pasture
3	19–20 Jan. 1949	Fine day	54·5*	46·5*	Hay in shed Kale fold Old pasture	8.0–10.0 10.0–12.15 12.15–2.45	Hay and mangolds fed in shed
4	17–18 Feb. 1949	Clear sunny day after frost	56·0	35·0	Silage in shed Old pasture	8.0–9.45 9.45–2.45	Hay and mangolds fed in shed
5	18–19 March 1949	Cloudy day	53·0*	44·0*	Silage in shed Old pasture	8.0–9.45 9.45–2.45	Hay and mangolds fed in shed
6	13–14 April 1949	Cloudy day and a little sun	64·0*	54·0	Silage in shed New ley	8.0–10.0 10.0–2.45	Hay fed in shed

* Shed temperature

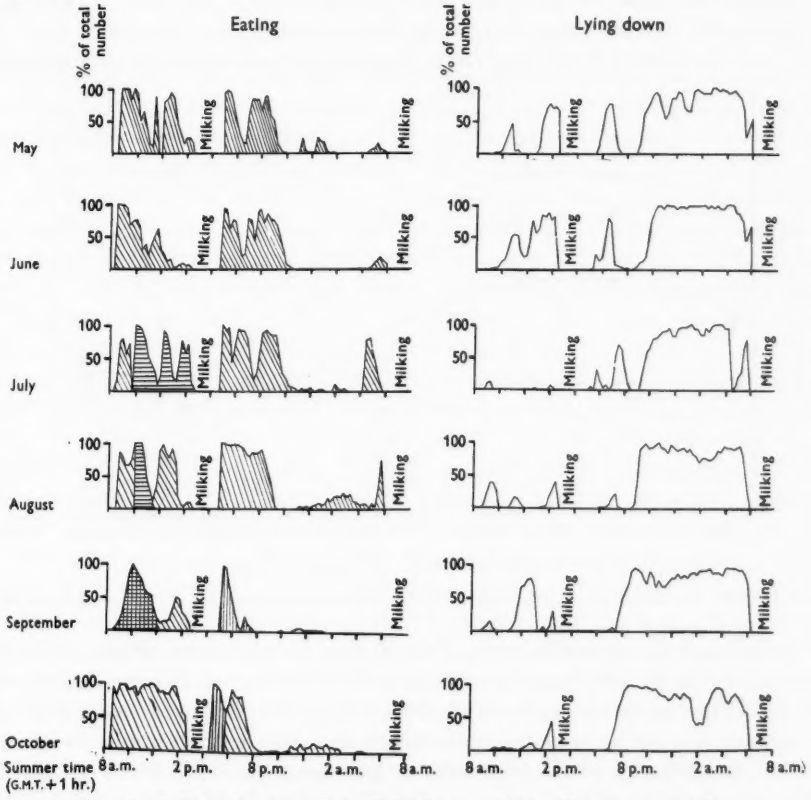
Table 5. *Details of the six 'summer' herd observations*

Observation no.	Date	Weather	Temperature (° F.) Max.	Min.	Feeding and management a.m.→p.m. milking	p.m.→a.m. milking
7	17–18 May 1949	Cool and dull, shower in a.m.	62·0	47·0	Folded on young ley	Same young ley
8	16–17 June 1949	Warm, sunny day, misty night	65·0	42·0	On same ley as before; herbage more mature	
9	8–9 July 1949	Fine and warm day	72·0	48·5	Cut lucerne fed on pasture	Ryegrass ley
10	12–13 Aug. 1949	Warm sunny day, fine night	72·0	47·0	Cut lucerne fed on pasture	Cocksfoot ley
11	26–27 Sept. 1949	Cloudy day, shower in a.m.	68·0	55·0	Grass silage fed on pasture	Hay fed on pasture
12	17–18 Oct. 1949	Bright day, shower in a.m.	60·0	46·0	Young ley	Hay fed on pasture

identical on the four recorded observations, and could in no way be attributed to any disturbance caused by the observer who worked in complete silence, and usually without any visible light.

Loafing. The two most important points about loafing have been dealt with indirectly in the two preceding sections. First, the characteristic period of loafing in the middle of the night when the cattle were at pasture; and secondly, the fact that when the cows were tied up in the cowshed at night approximately 50% of the time was spent loafing.

(*b*) *Summer management.* Details of the herd management, etc., are given in Table 5 and the results are presented graphically in Text-fig. 3 by the method described previously.



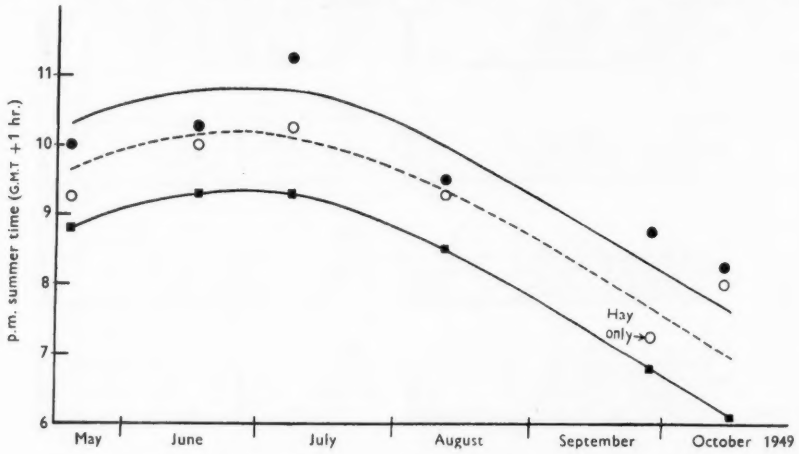
Text-fig. 3. The behaviour of a herd of dairy cows during the summer months.

Grass, ▨; silage, ▩; hay, ▥; lucerne, ▤.

Grazing. A pattern of behaviour, similar to that throughout the summer season, was discernible throughout the winter months. This pattern was identified by a grazing or eating peak in the morning immediately after the cattle entered the field, followed by a steady decline in numbers until the afternoon milking, and a similar single or double peak after the p.m. milking which tailed away by 10.0 p.m. Grazing after this time was spasmodic, although on four occasions a minor bout of grazing commenced shortly before the cows left the field for the a.m. milking. The grassland on which the herd was fed lucerne and

grass silage during the mornings of July, August and September was to all appearances quite bare and parched; yet from the graphs it can be seen that it provided a small amount of grazing for the herd. The 'grazing' in July consisted mainly of the animals picking up dead and wilted herbage which had been left after the pasture was topped over. Dead thistles which were quite dry were consumed by the cattle. Better pastures were generally provided for night grazing except in September, when even this field was barren and parched and the feeding of supplementary hay was necessary. Throughout the summer months the grazing during the evening was of a determined character, the cattle spending very little of their time in selecting the herbage or wandering long distances over the field.

To illustrate the effect of day length upon the behaviour of the herd, Text-fig. 4 has been constructed. All the times given are in British summer time (Greenwich mean time +1 hr.), and the points selected are (i) time of sunset, (ii) time when only 10% of the herd



Text-fig. 4. Relationship between time of sunset and behaviour of herd.

Time when 90% of herd laid down, ●—●; time when only 10% of herd grazing, ○ - - - ○; time of sunset, ■—■.

were grazing, and (iii) time when 90% of the herd were lying down. Points (ii) and (iii) were considered to be sound ones for comparing the behaviour on different dates, as they reflect a definite stage in the change of the behaviour pattern during the evening although a few animals may not be behaving as the remainder of the herd. It can be seen that the time when the majority of the herd stopped grazing and commenced to lie down was directly related to the time of sunset, and not, as far as could be observed, to the air temperature or the type of grazing available.

The distribution of the grazing as between day and night fields and factors affecting it could not be studied critically because of the feeding of supplementary foods at pasture. The general impression, however, from Text-fig. 3 would tend to confirm the four-cow results, that the distribution of grazing is about equal between the day and night paddocks on a time basis. In May and June, which were the only two months when the herd was on good leys day and night, 53% of the total grazing occurred between the a.m. and p.m. milkings in May and 43% in June.

Lying down. The lying-down curves (Text-fig. 3) are particularly interesting in that

a very similar and fixed pattern is noticeable throughout the summer season. This is marked by a major block of lying down from sunset to a time before the a.m. milking, with smaller and less noticeable peaks in the midst of the evening's grazing and after the morning's grazing. The period of lying down after the morning's grazing which was recorded in the May and June observations was not observed in July, and only to a lesser extent in August. This difference in behaviour was caused by flies, which on both occasions, and particularly in July, were observed to annoy the cattle greatly. The animals were prevented from lying down and spent this period of the day loafing in the shade trying to shake off the flies. The variation in the time at which the herd lay down at night is shown in Text-fig. 4, and considered in the previous section.

DISCUSSION

The two sets of observations described above indicate that the cow's natural inclination is to divide her total daily grazing time about equally between the day and night intervals between milkings. The data suggest that with the conditions of grazing observed, milking cows required a minimum grazing time of 3 hr. between milkings. The season was very dry, and at some of the summer observations the sward was sparse. There was some evidence, however, that with better herbage the grazing time might be substantially reduced, but under average conditions the cow giving 2–5 gal. of milk seems willing to graze the sward for 3 or 4 hr. in each interval between milkings. The evidence suggests that the practice of turning the herd on bare pasture at night, unless first given access to better grazing after the p.m. milking, will restrict the animals' intake of nutrients or require excessive travel in search of food. To avoid these difficulties, and assuming that grazing time is directly proportional to the animals' intake of grass dry matter, it would appear advisable to have the night and day paddocks of approximately equal size and with herbage of a similar quality. Similarly, in view of the clear-cut evidence of the general inclination of cows to rest during the hours of darkness, it would appear to be necessary to make special provision for grazing cattle during the season when the hours of darkness cover most of the interval between the evening and morning milkings. Without this the cow may have insufficient grazing time at night to satisfy its nutritive requirements.

As would be expected, cows grazing a more succulent sward drank less frequently than when on dry and more mature herbage. The number of drinks taken in the 24 hr. even under summer conditions was not, however, as great as might be expected and, indeed, the evidence suggests that, apart from a drink on reaching the field after the afternoon milking, water is not required during the night interval. Thus there may be little excuse for failure to use leys for herd grazing owing to lack of a water trough, if the ley is used at night and water offered on the way to the field.

A striking feature of the herd observations during the winter was the restlessness of the cows when tied on ordinary 5 ft. standings in a normal cowshed. This point is very clearly illustrated by the consistent failure of the cows to rest *en masse* when indoors at night during the period January to April, while in November and December nearly the whole herd remained lying for long periods. The 5 ft. concrete standings and metal yolks have been developed for sanitary reasons, but it seems clear that more thought should be given to the comfort of the cow. The covered straw yard may be the solution, but until these become available the data indicate that, under the conditions of our observations, the cows themselves would choose to be out of doors.

So far as the graphs indicate restlessness and discomfort, the importance of taking adequate precautions against the fly nuisance in the summer months is also clearly illustrated.

The data, however, are insufficient to venture to draw too many practical conclusions. They do, however, very clearly illustrate the pronounced tendency for a herd of cows to behave according to a very consistent pattern when environmental conditions are similar. It is also clear that by careful recording of the intimate behaviour of a small number of cows, or the mass observation of large numbers, useful information on the effect of management factors can be acquired.

SUMMARY

1. The behaviour of dairy cattle under normal commercial conditions of management was studied in two series of observations:

(*a*) Four cows were observed continuously for 24 hr. on four occasions at approximately two-weekly intervals.

(*b*) A herd (average fifty-two cows) was observed continuously for 24 hr. on twelve occasions at approximately monthly intervals.

2. Under our conditions and within our definitions, the cows grazed 6·52 hr., lay down 9·22 hr. and loafed 8·26 hr. on an average during 24 hr.

3. The cows ruminated 5·60 hr. per 24 hr.; 4·52 hr. when lying down and 1·08 hr. when loafing.

4. On average the animals defaecated 11·6 times, urinated 9·8 times, and drank 3·8 times in 24 hr.

5. One animal walked an average distance of 1·74 miles per 24 hr., excluding journeys to the cowshed.

6. No aspect of grazing behaviour was found to be strongly related to either current milk yields or air temperature.

7. When on 'good' pasture, as compared to 'poor', the animals grazed for a shorter time, walked less, and took fewer drinks.

8. From the results of the herd observations a similar periodicity of behaviour during 24 hr. was observed throughout the year but was more apparent when environmental conditions were similar.

9. When the herd was confined to 5 ft. concrete standings during the winter nights, the animals did not rest as comfortably as when out of doors.

10. Major peaks of grazing or eating were recorded after each milking followed by periods of lying down, especially during the night.

11. Time of sunset has been related to the time when grazing ceased and the herd lay down.

12. In view of the data collected, tentative suggestions for improving grazing management are made.

The authors wish to express their gratitude to the many helpers who willingly assisted in the collection of data often under unpleasant and rigorous conditions. To Mr D. Reid, late of the Grassland Improvement Station, Drayton, we are indebted for original advice and suggestions. We also wish to record our appreciation of the technical help given by Mr H. Burton, who assembled and maintained the infra-red ray equipment.

REFERENCES

(1) Edward, J. T. (1944). *Proc. Brit. Soc. Anim. Prod.* p. 95.
(2) Stapledon, R. G. (1948). *Agriculture*, **55**, 231.
(3) Stapledon, R. G. (1948). *Publ. Nat. vet. med. Ass.* no. 17, 14.
(4) Nichols, J. E. (1944). *Proc. Brit. Soc. Anim. Prod.* p. 66.
(5) Stoddart, L. A. & Rasmussen, D. I. (1947). *Circ. Utah agric. Exp. Sta.* no. 121.
(6) Cory, V. L. (1927). *Bull. Texas agric. Exp. Sta.* no. 367.
(7) Shepperd, J. H. (1927). *Bull. N. Dak. agric. Exp. Sta.* no. 211.
(8) Giöbel, G. & Lindbom, E. (1933–34). *Nutr. Abstr. Rev.* **3**, 1173.
(9) Johnstone-Wallace, D. B. & Kennedy, K. (1944). *J. agric. Sci.* **34**, 190.
(10) Grassland Improvement Station (1948). Stratford-on-Avon, Warwickshire. *Experiments in Progress*, p. 42.
(11) Grassland Improvement Station (1949). Stratford-on-Avon, Warwickshire. *Experiments in Progress*, p. 61.
(12) Hancock, J. J. & Wallace, N. M. (1947). *Proc. 7th Ann. Conf. N.Z. Soc. Anim. Prod.* p. 145.
(13) Hein, M. A. (1935). *J. Amer. Soc. Agron.* **27**, 675.
(14) Hodgson, R. E. (1933). *J. agric. Res.* **47**, 417.
(15) Atkeson, F. W., Shaw, A. O. & Cave, H. W. (1942). *J. Dairy Sci.* **25**, 779.
(16) Johnstone-Wallace, D. B. (1948). *J. Fmrs' Cl., Lond.* Part 1, 8.
(17) Hancock, J. J. (1948). Private communication.
(18) Moseley, T. W., Stuart, D. & Graves, R. R. (1929). *Tech. Bull. U.S. Dep. Agric.* no. 116, 9.
(19) Seath, D. M. & Miller, G. D. (1947). *J. Dairy Sci.* **30**, 921.
(20) Bonsma, J. C. (1940). *Fmg S. Afr.* **15**, 7.
(21) Seath, D. M. & Miller, G. D. (1946). *J. Dairy Sci.* **29**, 199.
(22) Bruce-Levy, E. (1935). *N.Z. J. Agric.* **50**, 140.
(23) Castle, M. E. & Foot, A. S. (1949). *Agriculture*, **56**, 1.

APPENDIX

By H. Burton and M. E. Castle

A description of the construction and use of portable infra-red ray equipment for animal observation in the field

In some of the herd observations previously described, use was made of infra-red ray equipment.

To observe a scene by infra-red rays it is necessary to have a source of intense radiation together with a means of receiving that part of the radiation which is reflected from the objects being viewed.

For the purpose of the tests, the source took the form of a motor-car headlamp fitted with a 48 W. 6 V. bulb. The glass was replaced by a filter which suppressed most of the light but which transmitted a considerable proportion of the near infra-red.

The type of receiver used was in the form of a telescope. The principles of receivers have been fully discussed by Pratt(1). The conversion of infra-red radiation into a visible picture is carried out by an infra-red image-converter tube. Briefly, this tube consists of a photo-emissive surface which emits electrons when subjected to infra-red radiation. These electrons are accelerated across a short gap by means of a very high voltage, and they then fall on a fluorescent screen. A visible picture appears on the screen corresponding to the pattern of the incident infra-red radiation. In addition to the tube itself, the telescope contains a quartz objective lens to focus the radiation on the photo-sensitive surface, and a conventional erecting lens and eyepiece for viewing the fluorescent screen.

To simplify the operation of the equipment, which had to be entirely portable, the headlamp was rigidly mounted to the telescope structure. The field of view under these conditions was limited by the width of the infra-red beam from the lamp, which was much more restricted than the field of the telescope. The lamp was therefore slightly de-focussed, and a perspex diffusing screen was added.

A voltage of about 6500 V. D.C. is required to operate the converter tube. This is obtained by means of a vibrator unit from a 6 V. D.C. accumulator. The current taken by the tube is only of the order of a fraction of a microampère, so that the output required from the accumulator to operate the vibrator is very small.

However, the accumulator in the equipment being considered had also to supply the headlamp, which required a current of 8 amp. and had to provide the supply for several hours. The accumulator had also to be carried by the observer, together with the vibrator and the telescope-lamp unit. As the best compromise between weight and available output, a semi-unspillable celluloid-cased cell of 33 amp./hr. capacity at a 20 hr. rate was selected. With intermittent use, sufficient capacity was available for one night's operations.

The vibrator and accumulator were carried in a haversack on the back, and a switch mounted on the telescope-lamp unit controlled the supply to both the lamp and the vibrator.

The different units of the equipment are shown in Pl. 1, and the equipment as ready for use is shown in Pl. 2.

The weights of the separate units are as follows:

Telescope lamp	$3\frac{1}{4}$ lb.
Vibrator	$6\frac{3}{4}$ lb.
Accumulator	11 lb.

This equipment, although valuable under certain conditions, had its limitations, particularly when observing a large number of animals as occurred in the herd observations (Part (*b*)). For watching, from a stationary position, not more than four animals at a range of 10–15 yards on a clear dark night, the equipment was ideal. When using it to observe a larger number of animals, either in the field or in the cowshed, the observer was liable to miscount the animals owing to the limited field of the screen. The constant wearing of the heavy equipment was also very tiring, and when misty, the lenses quickly clouded over.

REFERENCE

(1) PRATT, T. H. (1948). *Electronic Engng*, **20**, no. 247–8, pp. 274–8, 314–16.

(*MS. received for publication* 24 *March* 1950)

The units of the portable infra-red ray equipment.

The portable infra-red ray equipment as used in the field.

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424. A STUDY OF LACTATION AS AFFECTED BY HEREDITY AND ENVIRONMENT

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(With 2 Figures)

Since the existence of genes is recognized only through their effects, and since these effects must clearly be achieved through the mediation of some physiological process, it would seem more profitable, in studying the inheritance of milk yield, to differentiate and study the various components of the lactation yield than to attempt a direct determination of the exact number and nature of the genes involved.

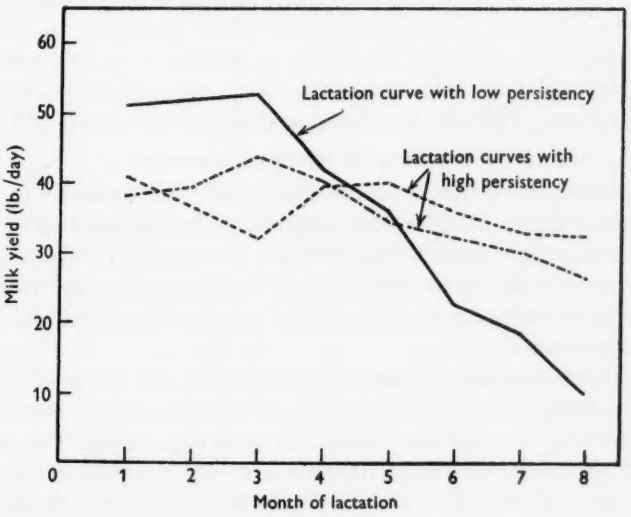

Fig. 1.

The lactation yield is not a single entity. In reality it represents the area of the lactation curve, which consists of two segments, (i) a rising segment, and (ii) a declining segment. Animals differ in the period during which the rise lasts, in the rate at which it occurs, and in the maximum level it attains. Differences are again found in the rate at which the decline occurs. The less 'persistent' animals decline in production very suddenly after the period of peak production, and rapidly 'dry off', whereas the more 'persistent' animals show little or no decline over a prolonged period (Fig. 1).

If high maximum yield and high persistency are distinct hereditary characters and are

* This paper has been condensed from a thesis with the same title, which was submitted by the author in 1940 for the degree of Ph.D. in the University of Glasgow. It was prepared for publication by Dr J. M. Rendel and Mr A. Robertson of the Animal Breeding and Genetics Research Organization, Edinburgh, whose work the author very gratefully acknowledges.

not physiologically incompatible, it should be possible to combine these in the same individual and thus obtain even higher yields. The validity of this contention can, however, only be determined by a study of the interrelationships of the different components of the lactation curve. This, in turn, must entail the differentiation of the effects of heredity from those of environment. Information regarding the latter point is supremely important, since only the genetic part of the variance of a character (Fisher (1)) can be transmitted from the parent to offspring, and therefore be stabilized by breeding. The present paper is devoted to a general study of these interrelationships, and the subject is dealt with in three separate parts. In the first part the maximum yield and persistency as components of the lactation curve are discussed, and in the remaining two sections the importance of environment and of heredity in determining the shape of the lactation curve are dealt with.

PART I. MAXIMUM YIELD AND PERSISTENCY AS COMPONENTS OF THE LACTATION CURVE

It should not be concluded that maximum yield and persistency are the only variable components of the lactation curve. Either or both of them may be dependent on more than one physiological function. Existing knowledge of the physiology of lactation is not, however, yet sufficiently complete to justify an attempt at a final resolution of the lactation curve into all its possible components. Until such information becomes available, the study of maximum yield and persistency may be undertaken as a first step.

1. REVIEW OF PREVIOUS METHODS

Before the shape of the lactation curve can be studied, it is necessary to give a quantitative expression to the shape. Of the two components of the lactation curve, i.e. maximum yield and persistency, the former is relatively easy to determine. Its value is given by the maximum ordinate of the curve. Persistency is more difficult to determine. It defines the slope of the curve and measures the relative* rate at which the maximum yield, when once reached, declines.

As early as 1886 Sturtevant (2) pointed out that each month's production is roughly a constant percentage of the production of the preceding month. In mathematical terms this means that the course of the lactation curve is exponential. This latter fact was independently pointed out by Brody, Ragsdale & Turner (3) in their study of the shape of the lactation curve of cows of various breeds. In order to describe the *average* lactation curve of a large number of cows of different breeds, they employed the equation

$$M_t = M_o e^{-kt},$$

where M_t is the milk production during any month t, M_o is the value of the initial theoretical rate of milk flow at the time of parturition (not attained in actual practice), k is the constant measuring the relative rate of decline, and e the base of natural logarithms. They found that the equation fitted the data remarkably well. On the basis of this work they formulated what they termed the law relating milk secretion with the advance of period of lactation, i.e. 'that each month's production after the second month is a constant percentage of the preceding month's production' (Turner, Brody & Ragsdale (4)).

Johannsen (5) has also used a ratio method, his measure of persistency being the ratio of the milk yield of the second 100 days of the lactation to that of the first 100 days. The drawback of this method is that the first 100-day period, which denotes the maximum

* For difference from 'absolute' rate see Fisher (6,7) and Brody (8).

physiological capacity of the cow for milk production, is too long to reveal differences between cows differing in their capacities to maintain yield during this crucial period. Two cows may give the same yield in the first 100 days of their lactation, yet they may have vastly different initial maximum yields, and therefore persistency values, for this period.

In a more recent study Pontecorvo (9) has used the exponential equation to determine the rate of decline. Instead of using the whole lactation curve, however, he has used what he terms 'the middle part of the lactation curve' to determine the persistency. He regards this portion as a distinct physiological component of the lactation curve, and holds that its use in place of the whole lactation curve constitutes a definite improvement in method.

It was concluded from a detailed study of all the relevant literature that the exponential equation probably provided the best measure of a lactation curve. In the preliminary studies detailed below this method was therefore adopted.

2. COLLECTION AND TABULATION OF DATA

The present study was based on the records of milk production of Ayrshire cows in six tuberculin-tested pedigree herds, all of which were situated in Ayrshire. The animals of five of these herds were of the 'milk' type and those of the sixth were of the 'vessel-bred' type.* The milk records used were those recorded officially by the Scottish Milk Recording Association, the yields being determined by the Association's own officials. The Association publishes the records of production of selected cows annually. In the past most of the work done on the inheritance of milk yield, both in this country and abroad, has been based only on such published records, and therefore on selected data. The results of such work are consequently of limited application. It was decided to avoid this criticism in the present study. Original milk record books were therefore obtained from the herd owners, and *all* normal records of milk production of the cows were abstracted. Preliminary examination of the records showed that, owing to the changes in policy in herd management, significant changes had taken place in the milk yields of cows during the period which followed the 1914–18 war as compared with earlier years. Although milk recording had been continuously carried on since 1910 in five of the six herds, it was decided to restrict the study to the years 1920–39. This selection was considered necessary in order to secure homogeneous data which would be as free as possible from the disturbing influence of changes in herd management.

Data regarding the number of records provided by each herd and the years to which these records belong are given in Table 1.

The milk records of the Scottish Milk Recording Association are based on measurements of milk yield which are carried out personally by the official Recorders, who visit each farm for the purpose once in every 14–28 days. The milk yield is determined by actual weighing over a period of 24 hr., and the results are entered in the milk records book. The day of visit is regarded as the middle day of the period covered by the test, and the total yield for the period is estimated by multiplying the observed yield by the number of days covered by the test. As the duration of this period may vary from visit to visit, the milk yield entries in the milk records book may represent varying periods. In the present study

* Until recently the Ayrshire breed consisted of two distinct types: (i) a 'vessel' type, the cows of which have tight, flat vessels and small corky perpendicularly hung teats, greater emphasis being laid on the conformation of the 'vessel'—udder—rather than the milk yield in this type, and (ii) a 'milk' type in which not so much attention is paid to the actual shape of the udder as to the milking propensities of the animal.

Table 1. *The number of records for each herd and the year to which these records belonged*

Herd	Before 1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	Total
A	45	13	18	22	24	26	25	29	29	27	24	27	23	26	23	24	26	26	28	—	485
B	—	—	—	1	17	25	32	32	28	35	30	33	31	44	31	36	37	31	34	1	478
C	3	1	2	2	3	42	61	55	45	47	65	64	56	62	62	67	71	61	12	—	781
D	—	—	—	—	—	—	—	—	—	4	18	25	30	31	32	38	36	35	25	—	274
E	—	—	—	—	3	11	26	25	43	39	30	51	45	46	54	49	62	50	33	—	567
F	12	8	10	12	19	16	20	17	20	20	15	18	20	16	8	5	4	3	—	—	243
Total	60	22	30	37	66	120	164	158	165	172	182	218	205	225	210	219	236	206	132	1	2828

Table 2. *Some typical examples of the fit of the exponential curve to the lactation curve*

(Nos. 1–4 are individual lactations; no. 5 is the average of 100 lactation curves.)

	Yield (lb. per day)									
	1		2		3		4		5	
Month of lactation	Observed	Estimated	Observed	Estimated	Observed	Estimated	Observed	Estimated	Observed	Estimated
1st	38·8	39·9	51·2	71·8	44·4	54·2	37·5	31·6	41·98	45·55
2nd	32·5	34·6	51·9	56·0	50·3	47·5	32·2	29·4	41·14	40·49
3rd	32·8	30·0	52·4	43·6	47·7	41·7	25·1	27·3	37·37	35·95
4th	28·3	26·0	42·3	34·0	40·1	36·6	23·2	25·4	33·35	32·00
5th	21·5	22·5	34·7	26·5	30·3	32·1	20·6	23·6	29·33	28·45
6th	19·4	19·5	22·4	20·6	31·3	28·2	18·8	22·0	25·77	25·29
7th	16·1	16·9	16·8	16·1	24·5	24·7	20·1	20·5	22·39	22·48
8th	14·1	14·6	8·5	12·5	19·2	21·7	19·5	19·0	18·85	20·03
9th	13·3	12·7	—	—	—	—	21·7	17·7	—	—
Σd^2	21·01		661·27		171·27		92·18		19·42	
Equation of fitted curve	$46{\cdot}01e^{-0{\cdot}14314t}$		$92{\cdot}15e^{-0{\cdot}24949t}$		$61{\cdot}82e^{-0{\cdot}13106t}$		$33{\cdot}98e^{-0{\cdot}07245t}$		$51{\cdot}24e^{-0{\cdot}11771t}$	

it was essential that the yields should be for periods of constant duration. After copying out the milk records, the yield for each successive 30-day period of every record was computed. It was observed that in many cases the first test was not made until about 30–40 days after calving. To overcome this difficulty the first 15 days of every record were omitted when fixing the limits of the different periods. The computed yield for the first 30-day period thus covered from the fifteenth to the forty-fifth day after calving.

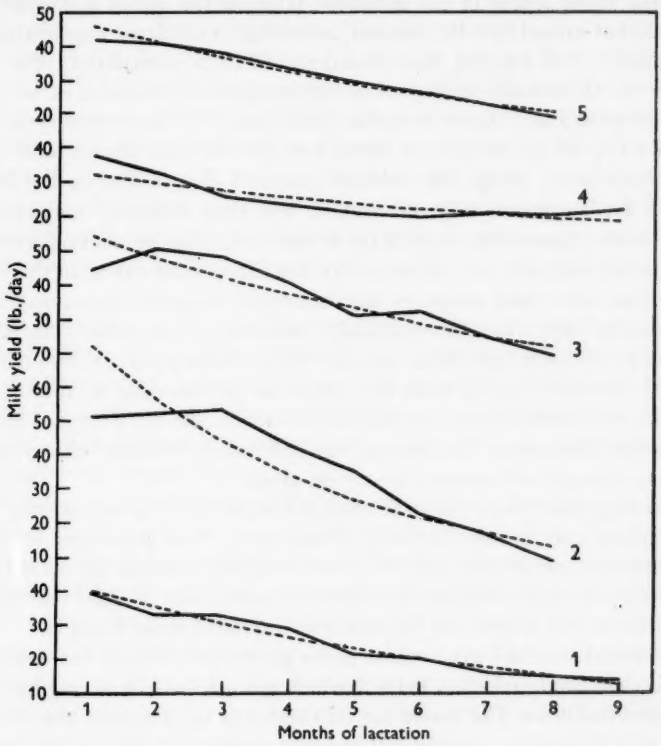

Fig. 2.

3. FIT OF THE EXPONENTIAL CURVE

In the herds under study the average calving interval was found to be 12 months. Since the average gestation period of cows is 280 days, this indicates that the cows were served approximately 3 months after calving. The work of Gavin (10), Sanders (11,12) Gaines (13) and Østergaard (14) has shown that gestation appreciably affects the milk production some 5 months after conception. To obviate the necessity of correcting for the influence of varying service periods, it was decided to use only that part of the lactation curve which was free from the influence of pregnancy.

The process of curve-fitting was straightforward. The method described by Kartha (15) was adopted, the final value of persistency being expressed on a percentage basis, i.e. $100 \times e^{-k}$, for each curve. As a result of this preliminary work of curve-fitting it was soon found that, whereas the exponential equation gave a reasonably good fit with some lactation curves, the fit was poor in others. Table 2 and Fig. 2 show the fits for some

selected curves (nos. 1–4), which are typical of the general results obtained, and also for an average lactation curve (no. 5).

As will be observed by a comparison of the observed with the estimated yields given in the table, the fit is fairly close with curve 1, though it is poor with the remaining three individual curves. This fact is confirmed by reference to the d^2 values given for each curve. In particular, the estimated yields of curve 2 diverge widely from the observed yields throughout the whole course of the lactation. Whereas the actual maximum daily yield of this cow did not exceed 52·4 lb., she had, according to the fitted exponential curve, the capacity to yield 71·8 lb. 1 month after calving and 92·15 lb. immediately after parturition. Undoubtedly the theoretical curve gives a very exaggerated estimate of her capacity for maximum milk production. Curve 4, on the other hand, is of the reverse type. The yield of this cow according to the theoretical curve was 34·0 lb. immediately after calving and 31·6 lb. a month later, though she actually produced 37·5 lb. during the latter period. Her capacity for maximum milk production was thus definitely understated by the exponential curve. Again with curve 3 the actual maximum production was obtained in the second period, and not, as is indicated by the exponential curve, in the first.

It will be clear from these examples that individual lactation curves may not conform to the exponential type even approximately, and that, if the latter type of theoretical curve is used to represent such data, one may fail to distinguish real differences between the shapes of the curves—even when the curves are as dissimilar as the 'observed' and 'estimated' curves shown as no. 2 in Fig. 2. No doubt, the no. 2 type of curve exhibits a rather extreme divergence. In general, the differences between 'observed' and 'estimated' values, though well marked, are not so great.

It has been suggested that a better fit can be obtained with the exponential curve if the declining segment only is used in fitting (Turner (16)). This procedure does not appear justifiable, however, since it not only fails to distinguish between curves with rising segments of varying duration, but also involves the comparison of these curves for varying periods. Moreover, the fit will still be poor with curves of types 2 and 4.

It may be noted that the exponential curve gave a good fit for the *average* lactation curve. This is clear from curve 5 in Table 2, which was obtained by averaging 100 lactation curves of individual cows. The reason for this better fit is, of course, that the differences of curves 2 and 4 from the exponential are compensating in nature. The fact that the exponential curve gives a good fit with *averages* of lactation curves is no proof that the rate of decline of milk yield with the advance of lactation is normally exponential.

4. FIT OF THE PARABOLIC EXPONENTIAL CURVE

In view of the considerable discrepancies which were found to exist between the actual lactation curves and the fitted exponential values, an attempt was made to see whether some other type of mathematical curve would represent the lactation curve better. As the course of the latter is parabolic, a trial of the parabolic exponential curve, $y = Ae^{bt+ct^2}$, was suggested by Robb (17). This was studied by means of data secured from the Institute's own herd. One hundred lactation records were taken, and the milk yield of each worked out for successive 28-day periods. Unlike the previous data these records were based on the day-to-day recording of the milk yields of each cow throughout her lactation. As the fitting is carried out in two stages it is possible to examine separately the fit of the linear and the parabolic equations to the data, and to judge whether the contribution of the

parabolic constant is significant in the reduction of variation. Where the parabolic term is significant it shows that the linear constant alone does not account for all the systematic variations of the data, i.e. that the exponential curve does not give the best possible fit.

This work confirmed the previous finding that the goodness of fit of the linear term (i.e. its contribution in the reduction of sum of squares) varied considerably in the individual records. The sum of squares which the linear term accounted for varied from 33·92 to 94·48% of the total in individual curves, the mean being $85{\cdot}06 \pm 1{\cdot}43$% and the standard deviation $14{\cdot}28 \pm 1{\cdot}01$%. This contribution was significant at 1% level in ninety-one curves, not significant at 1% but significant at 5% level in another four curves, and not significant even at 5% level in the remaining five curves. The milk yields of successive periods in these five lactations were too erratic to be satisfactorily graduated by any simple mathematical curve. The equation gives, of course, a value of b (which is a measure of the average slope of the curve) for each of these lactation curves, but owing to the highly erratic slope at different points of these curves, this average value has little meaning. In the remaining records the linear term is undoubtedly the preponderating term and represents the greater part of the systematic variation of rate of milk production with advance in lactation.

The above facts do not indicate, however, that in these records the exponential curve gives as good a fit as it is possible to obtain, for, if this were so, the contribution of the additional constants to the reduction of variation would be insignificant. It was, in fact, observed that in forty-eight of the 100 lactations the parabolic term brought about a significant reduction in the sum of squares—in twenty-three cases significant at 1% level and in the remaining twenty-five at 5% level.

From the foregoing it will be clear that the shape of the lactation curve of individual cows conforms more closely to the parabolic exponential than to the exponential type. The use of the parabolic exponential is therefore to be preferred in graduating data for any genetical study of the shape of the lactation curve. Apart from the advantage of the better fit, the parabolic exponential curve provides two separate constants—the linear, which measures the average slope of the curve, and the parabolic, which describes how on an average the rate of this slope varies from period to period. In any study of persistency it is clearly of advantage to be able to separate these two constants, so that they can be correlated independently with any relevant environmental or hereditary factors.

5. THE FREQUENCY DISTRIBUTION AND CONSTANTS OF THE VARIOUS CHARACTERISTICS OF THE LACTATION CURVE

In view of the results reported in the previous section it was decided to use the parabolic exponential curve to graduate the data of this investigation. However, by this time the exponential curve had been fitted to nearly the whole of the data of one herd. The parabolic exponential curve was therefore fitted to the data of the remaining herds only, comprising 1900 records.

A total of 2828 milk records derived from 863 different cows were tabulated. Of these 863 cows, 215 had only one record each. For the remaining 648 animals two or more records were known. It was noticed that there was a far smaller variability in the persistency of different cows on the basis of their heifer than their later records. It was therefore decided to exclude all cows which had only one record. This procedure left 2613 records, 2392 of which came from herds A–E ('milk' type), and the remaining 221

from herd F ('vessel' type). It was considered advisable to keep the 'milk' herds data separate from the 'vessel' herd data, as there are considerable differences in the systems of management of these two types of herds. The study of the environmental factors were made from the 'milk' herds only. The main statistical constants of these data are given in Table 3.

Table 3. *The statistical constants of the 'raw' data used in the present studies*

	Total milk yield	Maximum yield	Persistency	Parabolic constant
Mean	8133·6±35·23	46·28±0·101	90·08±0·201	−0·00370±0·00014
Standard deviation	1723·0±24·91	9·84±0·142	4·95±0·072	0·00584±0·00010
Coefficient of variation	21·18±0·325	21·27±0·319	5·45±0·08	157·84±0·66
μ_2	47·50	96·91	24·54	136·44
μ_3	+135·87	+333·42	−50·30	−730·38
μ_4	+7708·30	+29328·53	+1908·43	69316·38
β_1	0·17	0·12	0·17	0·21
β_2	3·42	3·12	3·17	3·72
$\gamma_1=\sqrt{\beta_1}$	+0·42	+0·35	−0·41	−0·46
$\gamma_2=\beta_2-3$	+0·42	+0·12	+0·17	+0·72
Skewness	+0·19	+0·18	−0·22	−0·18
Mode	7808·1	44·993	91·170	0·00478

It will be observed that the distribution of all the four constants is slightly asymmetrical. The mode is higher than the mean in the case of persistency and parabolic constant, and lower than the mean in the case of maximum yield and total yield. The skewness is negative for persistency and parabolic constant and positive for maximum yield and total yield. There exist very great differences between the variability of parabolic constant and persistency as compared with total yield and maximum yield. The value of the coefficient of variation is lowest for persistency and highest for the parabolic constant.

PART II. THE LACTATION CURVE AS AFFECTED BY ENVIRONMENT

The observed differences between the production of individual cows may be partly hereditary and partly environmental. For the correct estimation of the former, it is important to allow for the obvious effects of the latter. The nature of food supply is bound to differ from year to year, and still more in different seasons of the same year. When the influence of an environmental factor is known, it is possible to 'correct' or 'standardize' the record to what it would have been under the conditions of the standard environment. The determination of such 'correction' factors is a prerequisite to all genetical researches. Of the various environmental factors, feeding and management are perhaps the most potent in determining differences in the production of individual animals. It has not been possible, however, to examine the influence of these factors statistically in the present investigation, since the differences in this respect between individuals and even between herds are not known.

In the present investigation all the cows were milked twice daily, so that there were no differences in this respect between the individual records. The segment of the lactation curve used was restricted to the first $8\frac{1}{2}$ months, and the average service period was about 3 months. It was not possible to study the influence of the dry period, since, owing to the prevailing practice of stopping the recording of milk of certain animals with the close of the calendar year, the length of this period could not be correctly ascertained in all records. Only two important factors remained for investigation, i.e. month of calving and age. The

influence of these factors was therefore studied, using the total data of the five 'milk' herds (2392 records), and is detailed in the following sections.

One further point should be mentioned. Table 4 shows the number and the ages of the cows which calved in each month of the year. It will be observed that the proportion of animals calving in different months varied markedly. In general, many more cows calved during the spring and autumn than during the summer.

Table 4. *The variation of month of calving with age in lactations*

Month of calving	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	Total no. of cows	Percentage of total no.	Mean age in lactations
Jan.	67	64	52	47	19	13	7	2	2	2	1	1	—	277	11·58	3·03
Feb.	50	74	55	43	30	17	9	11	3	4	1	—	—	297	12·42	3·42
Mar.	39	89	89	70	70	34	23	14	6	—	2	1	—	437	18·27	3·84
Apr.	24	76	68	56	32	30	19	16	7	7	3	3	—	341	14·25	4·13
May	16	23	25	15	22	12	8	5	7	4	3	—	—	140	5·85	4·36
June	11	8	4	3	5	8	5	4	1	2	1	—	1	53	2·22	4·60
July	3	5	7	4	1	2	2	2	3	—	—	1	—	30	1·25	4·53
Aug.	7	10	8	7	6	6	5	1	1	1	1	—	—	53	2·22	4·11
Sept.	72	22	17	10	11	3	4	1	1	1	1	—	—	143	5·98	2·42
Oct.	111	49	25	10	3	6	4	4	5	3	1	—	—	221	9·24	2·36
Nov.	80	53	21	19	7	12	6	3	4	2	1	1	—	209	8·74	2·76
Dec.	54	60	32	12	15	8	6	3	—	1	—	—	—	191	7·98	2·71
Total	534	533	403	296	221	151	98	66	40	27	15	7	1	2392	100·000	3·39

The important point is, however, that a distinct correlation existed between the age of the cow and the month in which she calved. This is clear from the last column of Table 4, which gives the mean age of the cows calving in each month. It will be observed that the means are lowest in the autumn months and are highest in midsummer, the seasonal trends being very regular and consistent. A very much higher proportion of younger cows calve, therefore, in the autumn and winter than in the spring or summer.

1. GENERAL METHOD OF STUDY

In studying the influence of the month of calving and of age on the different constants of the lactation curve, it is necessary to allow for the correlation reported in the preceding paragraph. The method of multiple regression would be ideal for the purpose, if the regressions were linear. However, as will be shown later, the regressions are distinctly non-linear. This method is therefore not practicable.

It was found that in the data available for the present study there was a number of animals which calved in the same month during two or more successive lactations. The variation of the different constants of the lactation curve with age was determined from the data furnished by such animals by the 'paired-lactation' method, the details of which will be explained later. Such constants will be free from the influence of month of calving. The influence of month of calving was then studied from the corrected records, and correction factors for month of calving worked out. These were in turn applied to the original data, and from the data corrected in this way the effect of age was studied.

The variation of the parabolic constant was studied from the raw data, i.e. without allowing for the correlation observed between month of calving and age.

Unless otherwise stated the constants of the lactation curve are defined throughout the present paper as follows: (i) *persistency* is the linear constant of the exponential curve expressed on a percentage basis, and represents the average relative slope of the lactation curve; (ii) *the parabolic constant* is the third term of the parabolic exponential equation,

and represents the rate of change of decline rate; (iii) *the maximum yield* is the highest 30-day yield calculated from the official milk records; and (iv) *the total yield* is the yield of milk for the portion of the lactation curve studied (i.e. from 15 days to 8½ months after calving).

2. INFLUENCE OF MONTH OF CALVING

Variation of the constants of the lactation curve with the month of calving

The variation in the constants of the lactation curve with month of calving is shown in Table 5. The analysis of variance of the various constants with month of calving gave significant variance ratios in every case. The proportion of the total variance which was due to variation between months is given in Table 6.

Table 5. *The variation of the different constants of the lactation curve with month of calving*

Month of calving	Persistency (%)	Parabolic constant	Maximum yield (lb.)	Total yield (lb.)
Jan.	90·85±0·265	−0·00338±0·00030	45·76±0·485	8323·5±90·5
Feb.	89·43±0·284	−0·00607±0·00036	45·03±0·443	7843·9±90·9
Mar.	88·88±0·216	−0·00757±0·00032	45·94±0·401	7940·2±78·8
Apr.	88·16±0·239	−0·00616±0·00036	46·80±0·400	7800·9±79·4
May	87·78±0·372	−0·00301±0·00048	48·34±0·753	7837·5±138·1
June	88·20±0·570	−0·00161±0·00077	48·63±1·233	7775·9±216·5
July	90·23±0·918	−0·00163±0·00080	44·23±0·986	7525·0±214·9
Aug.	91·58±0·638	+0·00036±0·00096	43·12±0·882	7634·4±194·3
Sept.	91·30±0·323	+0·00069±0·00043	45·13±0·646	7836·5±127·5
Oct.	91·64±0·269	+0·00102±0·00037	45·79±0·532	8119·4±108·3
Nov.	91·97±0·267	+0·00033±0·00039	45·76±0·557	8224·3±108·7
Dec.	91·11±0·260	−0·00124±0·00039	46·02±0·611	8176·1±122·4
Mean of all months	89·92±0·092	−0·00370±0·00014	45·98±0·164	7964·3±32·2

Table 6. *The variance due to month of calving*

Constant	Variation due to months (%)
Persistency	9·57
Parabolic constant	10·13
Maximum yield	1·05
Total yield	0·73

It is obvious from this table that the month of calving was only of minor importance in the variation of total yield. This was due largely to two causes:

(i) The variation of persistency and maximum was roughly inverse, i.e. those months with a high persistency had a low maximum and vice versa.

(ii) The number of calvings was lowest when the yield was lowest.

The validity of the above results might be questioned on two grounds:

(*a*) that other environmental factors were not random from month to month,

(*b*) that there may have been a tendency for the best and poorest yielding cows to calve at different times of the year.

It was not possible to test the first objection, and it was assumed that none of the other environmental factors (e.g. service period or dry period) except age was correlated with month of calving. The variations of the separate constants of the lactation curve were then studied afresh by comparing the age-corrected records *of the same cow* falling in different calving months. Such intra-cow comparisons should preclude the possibility of objection (*b*) above affecting the observed differences.

By this method a series of comparisons of cows calving in January with their lactations in other months was obtained and so on. Then all these sets of comparisons, having been found to be in reasonable internal agreement, were averaged together to give a final comparison between months of calving not complicated by differences between cows. Table 7 shows the mean values for the different months, taking January as a standard with an arbitrary value of 100.

Table 7. *The effect of month of calving on persistency, maximum yield and total yield, the values for January being taken as* 100

	Persistency	Maximum yield	Total yield
Jan.	100	100	100
Feb.	99·62	99·16	97·95
Mar.	98·44	101·10	97·43
Apr.	97·00	103·41	96·28
May	96·11	105·63	95·26
June	96·46	106·78	94·60
July	97·63	100·10	91·04
Aug.	102·18	95·35	95·76
Sept.	100·49	99·80	95·26
Oct.	100·96	99·96	99·44
Nov.	101·50	98·78	100·61
Dec.	99·83	99·28	98·08

It will be seen that cows calving in the spring months had a high maximum yield coupled with a low persistency and a low total yield. This can be related to the herbage conditions under normal conditions of management. Watson (18) showed that the maximum growth of grass takes place in May and then diminishes rapidly until July. There is a second peak in September, and then the growth falls off for the winter months. Those cows calving in the spring get good grazing at the start, but it deteriorates rapidly and results in a low persistency figure. Cows calving around November and December get the spring grass towards the middle of their lactations and therefore show a higher persistency.

It is clear that the general trend of persistency is in the opposite direction to that of maximum yield, so that their effects on total yield tend somewhat to neutralize each other. It is worth noting that during the period when total yields were lowest the number of cows calving was also lowest.

3. INFLUENCE OF AGE

(*a*) *Variation of constants with age*

The influence of the month of calving on the three constants of the lactation curve, i.e. persistency, maximum yield and total yield, was discussed in the last section, and correction factors were given which enabled these to be standardized for differences in the month of calving. The influence of age was next studied from the data thus standardized. The present section reports the results of this study. Unfortunately, the time available to the writer did not permit him to undertake a full study of the significance of the parabolic constant in relation to the inheritance of persistency.

An analysis of variance of the constants of the lactation with age showed that the percentages of the total variation due to age were as shown in Table 8.

Table 8. *Variance due to age in lactation*

Constant	Proportion of variance due to age (%)
Persistency	17·78
Maximum yield	31·16
Total yield	14·67

The lower variation of total yield with age is again due to the inverse relationship of persistency and maximum yield, the one decreasing and the other increasing with age. In all cases the regressions of the constants on age were significantly non-linear.

There are two possible ways of evaluating the corrections for age. One may take all lactations available and work out corrections for them. This has the disadvantage that the older age groups will definitely contain selected cows. This can be termed the 'lumped-lactation' method. On the other hand, one can compare the first lactation with the second by considering only cows having two or more lactations, and so on, which will eliminate a large part of the error due to selection. Table 9 shows the values obtained by the two methods for persistency, maximum yield and total yield.

Table 9. *The variation of persistency, maximum yield and total yield with age*

	Comparative values					
	Persistency of 4th lactation = 100		Maximum yield of 4th lactation = 100		Total yield of 4th lactation = 100	
Age in lactations	'Paired-lactation' method	'Lumped-lactation' method	'Paired-lactation' method	'Lumped-lactation' method	'Paired-lactation' method	'Lumped-lactation' method
1	106·64	105·25	71·81	73·07	85·05	82·37
2	101·74	101·64	88·86	90·19	92·89	90·04
3	100·28	100·16	96·41	97·36	97·70	95·84
4	100·00	100·00	100·00	100·00	100·00	100·00
5	99·83	100·52	97·69	101·12	100·31	102·74
6	99·49	101·25	96·80	101·94	99·15	104·29
7	99·12	101·71	95·91	102·54	97·06	104·87
8	99·04	102·22	94·77	102·40	94·54	104·70
9	99·34	102·28	93·01	101·10	92·13	104·02
10	99·67	102·17	90·90	98·76	90·34	103·03
11	98·88	102·17	89·91	96·82	89·70	101·98

The figures show that persistency dropped sharply after the first lactation and then continued to decrease more gradually as the cow got older. The apparent increase at the higher ages appeared to be due to selection, as it did not show up in the 'paired lactations' column. These results are in agreement with those of Turner (19), Pontecorvo (9) and others.

Maximum yield increased until the fourth lactation and thereafter decreased fairly rapidly. Here again the 'lumped-lactation' figures continued to rise and showed a maximum at about the seventh lactation. The variation of maximum yield with age has been studied by many workers in the past. Sanders (20) found that the maximum increased up to 6·58 lactations (by the 'lumped-lactation' method) and 6·88 lactations (by the 'paired-lactation' method) and then decreased, and that the mature maximum yield was 68% higher than that of the first lactation.

The figures for total yield show a maximum at the fifth lactation with a decrease thereafter. Here again, the 'lumped-lactation' method shows a rise until much later with a maximum at seven lactations. The variation of milk yield with age has been frequently studied, but in most studies the 'lumped-lactation' method has been used. Several investigations have been made previously on Scottish Milk Recording Association data, amongst others those of Tocher (21), Kay & McCandlish (22) and Glen & McCandlish (23) may be mentioned. Tocher studied only the records for the years 1911–12 and found a maximum at 12–13 years, doubtless due to his omission to allow for selection. Kay & McCandlish (22) and Glen & McCandlish (23) studied Ayrshires, using only those cows which had com-

pleted at least five lactations. Some of their results are directly comparable with those of the present study and are shown in Table 10. This agreement is reasonably close, although the present data indicate a more rapid approach to maturity than the earlier data.

Table 10. *The effect of age on the relative total yield as shown by Glen & McCandlish* (1930) *and also by the present work*

	Relative total yield (5th lactation = 1·00)	
Age in lactations	Glen & McCandlish	Present data
1	1·16	1·18
2	1·13	1·08
3	1·08	1·03
4	1·02	1·00
5	1·00	1·00

(*b*) *Repeatability of the lactation curve*

Owing to the variation of environmental factors from year to year, the correlation between succeeding lactations of the same cow is not perfect. It is, however, of interest to calculate the repeatability of the various constants of the lactation curve.

Correlations between the constants in the various lactations are shown in Table 11 for persistency and in Table 12 for maximum yield and total yield. In order that the significance may be judged, the numbers of cows on which the correlations were calculated are included in Table 11 and were the same for the data in Table 12. Of the correlation between the first and other lactations for persistency only those up to the fourth lactations were significantly different from zero. The values of r were highest for successive lactations and diminished as the period of time between the records increased. The correlation between successive lactations did not show any change with age up to six lactations. Similar results were obtained for maximum yield and total yield (Table 12), although the decline in the correlation coefficient with time between lactations was not so marked. Maximum and total yield were rather more repeatable than persistency, the average figures for the correlations between succeeding lactations being 0·49, 0·65 and 0·70 for persistency, maximum yield and total yield respectively. Variations during the productive lifetime of the cow therefore played a considerable part in the total variation. The decrease in correlation with time may have been due to semi-permanent changes in herd management. It is probable therefore that the prediction of any given lactation can be carried out much more accurately by considering the one immediately preceding it than by any other.

It is of interest to see whether the prediction of a future record is greatly improved by considering other records than the one immediately preceding it. This is possible by the technique of multiple regression from which a prediction equation can be obtained in terms of any number of other lactations. For this study the 221 cows possessing records for the first four lactations were used and the correlation coefficients worked out for this sample. Then the multiple correlations were evaluated for the fourth lactation in terms of the others. The results are shown in Table 13.

The last three figures in the table represent the accuracy of prediction of the fourth lactation from the third, and also from the second and third, and from all of the first three lactations. It will be seen that when the yield of the third lactation was known, there was

Table 11. *Correlation between persistencies at different ages*

(Lower left—correlation coefficient; upper right—number of cows used to determine the correlations.)

Heavy type denotes not significantly different from zero

Age in lactations	Age in lactations 1	2	3	4	5	6	7	8	9	10
1	—	503	363	256	180	114	61	34	19	15
2	+0·47	—	363	262	180	118	68	43	26	17
3	+0·33	+0·50	—	270	190	121	69	45	23	15
4	+0·25	+0·44	+0·50	—	204	131	78	52	28	18
5	**+0·08**	+0·31	+0·43	+0·49	—	139	84	56	31	19
6	**+0·08**	**+0·12**	+0·26	+0·39	+0·54	—	92	56	34	21
7	**+0·18**	**+0·03**	+0·31	+0·30	+0·34	+0·50	—	64	36	23
8	**+0·14**	**+0·00**	**+0·16**	**+0·17**	+0·42	+0·34	**+0·20**	—	37	22
9	**−0·21**	**−0·39**	**−0·04**	**+0·16**	**+0·13**	**+0·22**	+0·22	+0·41	—	26
10	**−0·20**	**−0·13**	**+0·15**	**+0·26**	**−0·16**	**+0·25**	+0·63	+0·44	+0·66	—

Table 12. *Correlation between yields at different ages*

(Lower left—maximum; upper right—total yield; numbers of cows as in Table 11.)

Heavy type denotes not significantly different from zero

Age in lactations	Age in lactations 1	2	3	4	5	6	7	8	9	10
1	—	+0·74	+0·68	+0·52	+0·48	+0·37	+0·62	**+0·33**	**−0·05**	**−0·03**
2	+0·69	—	+0·73	+0·64	+0·55	+0·38	+0·48	+0·52	**+0·01**	**−0·09**
3	+0·61	+0·68	—	+0·72	+0·60	+0·52	+0·58	**+0·30**	**+0·12**	**+0·06**
4	+0·48	+0·57	+0·64	—	+0·73	+0·56	+0·51	+0·30	**+0·03**	**+0·02**
5	+0·49	+0·49	+0·55	+0·65	—	+0·64	+0·55	+0·46	**+0·23**	**+0·26**
6	+0·41	+0·30	+0·45	+0·52	+0·67	—	+0·55	+0·26	**+0·25**	**+0·29**
7	+0·68	+0·59	+0·61	+0·48	+0·60	+0·52	—	+0·53	**+0·08**	**+0·34**
8	**+0·33**	**+0·30**	**+0·18**	**+0·14**	+0·34	+0·32	+0·54	—	+0·40	+0·43
9	**+0·01**	**−0·01**	**+0·34**	**+0·27**	+0·38	+0·37	**+0·26**	**+0·23**	—	+0·76
10	**+0·40**	**+0·23**	**+0·26**	**+0·27**	**+0·40**	**+0·43**	+0·54	+0·63	+0·77	—

Table 13. *Repeatability of total yield and persistency*

Correlation coefficient (the numbers represent the number of lactations concerned)	Total yield	Persistency
$r_{1,2}$	0·7623	0·4529
$r_{1,3}$	0·7154	0·2493
$r_{1,4}$	0·5265	0·2386
$r_{2,3}$	0·7655	0·5876
$r_{2,4}$	0·6546	0·4873
$r_{3,4}$	0·7226	0·5648
$R_{4\,(3,2)}$	0·7397	0·5965
$R_{4\,(3,2,1)}$	0·7430	0·5966

little gain in considering the production of the earlier lactations in estimating the fourth lactation. The regression equations were as follows:

(i) total yield: $Y_4 = 1647{\cdot}7 - 0{\cdot}14\,Y_1 + 0{\cdot}35\,Y_2 + 0{\cdot}62\,Y_3$.

(ii) persistency: $Y_4 = 23{\cdot}69 + 0{\cdot}05\,Y_1 + 0{\cdot}27\,Y_2 + 0{\cdot}41\,Y_3$.

(c) *Correlation between the different constants*

After correction of the data for the two environmental factors, month of calving and age, the relationship between the constants was studied. As these data were obtained from several herds, the overall correlation is a compound one, being made of three parts: (i) between herds, (ii) between cows within herds, (iii) between lactations within cows.

A method for the separate determination of these components is available in the analysis of covariance. It should be noted that some of the past studies have not taken this into account. For instance, both Gaines (24) and Gooch (25) reported a negative correlation between maximum yield and persistency, although neither of these authors showed whether this correlation existed between the records of different cows as distinct from a negative correlation between the various records of the same cow. A detailed analysis of variance was carried out for all the three constants and the correlations are shown in Table 14.

Table 14. *Correlations between persistency and maximum yield, maximum and total yield, and persistency and total yield*

	Correlation		
Component	Persistency and maximum yield	Persistency and total yield	Maximum and total yield
Overall	−0·08	+0·40	+0·81
Between herds	−0·63	−0·49	+0·96
Within herds	−0·07	+0·42	+0·80
Between cows within herds	0·00	+0·46	+0·86
Within cows	−0·17±0·02	+0·37	+0·70

The following facts should be noted:

(i) *Persistency and maximum yield.* The 'between herds' value was not significantly different from zero. The results thus show that the negative correlation between maximum yield and persistency was really due to the 'within cows' effect.

(ii) *Persistency and total yield.* Again the 'between herds' value was not statistically significant, but there was a significant correlation within herds, between cows and within cows.

(iii) *Maximum and total yield.* The correlation coefficients were all high and highly significant and show the importance of maximum yield in determining total yield. In every case the correlation between maximum and total yield was significantly higher than the corresponding correlation between persistency and total yield.

(d) *Relative importance of maximum and persistency*

The relative importance of maximum and persistency in determining total yield may now be determined. There is no doubt that the relative influence varies from herd to herd, but here only the overall picture is considered. This may be measured from Wright's path coefficients (26) which in this simple case reduce to the partial regression coefficients. It is clear from the results shown in Table 15 that the maximum yield is much more important than persistency in determining total yield, and that the two together determine total yield almost completely.

Table 15. *Relative importance of maximum yield and persistency in determining total yield*

	Within herd	Between cows	Within cows
Standard regression coefficients:			
Maximum yield – total yield	0·834	0·863	0·779
Persistency – total yield	0·480	0·460	0·502
Relative value* of maximum yield and persistency	3·02	3·52	2·40
Multiple correlation of total yield with maximum yield and persistency	0·939	0·978	0·854

* Obtained from the squares of the two preceding values in the same column.

PART III. THE LACTATION CURVE AS AFFECTED BY HEREDITY

Numerous researches have been directed in the past to the study of the mode of inheritance of milk yield. The general view is that the mode of inheritance is Mendelian, and that it is, like any other quantitative character, of the multiple-factor type.

Another important aspect of the inheritance of milk yield is the degree to which observed differences in individual animals are hereditary as distinct from environmental. If, for instance, the total variance of milk yield observed in any population is represented by σ^2* and the parts of this variance due to hereditary and to environmental causes by σ_H^2 and σ_E^2 respectively, it is not yet known what is the value of σ_H^2/σ^2. There can be no doubt that such knowledge would prove most valuable to the herd owner, in that it would enable him to estimate what improvement might be expected as a result of the action of a certain selection differential. In the absence of such information he is inclined to believe that *all* the observed differences between his animals are genetic. Accordingly, he mates the best animal to the best, only to find the progeny may show distinct regression. The cause of such regression lies largely in the fact that, in reality, only a part of the observed superiority of the parents is due to genetic causes, i.e. the progeny only inherit that part (σ_g^2) of the selection differential which is genetic.

It is clear that the value of σ_H^2 will vary from population to population, and that in any given population it can be changed either by breeding or by altering the environment. For instance, continuous inbreeding will, within a few generations, reduce the genetic variability considerably, thus increasing the proportion of σ_E^2. This point is well demonstrated by Wright's work with guinea-pigs (27).

When the present study was made no work on the relative roles of heredity and environment in the variation of milk yield of Ayrshire cows had been reported in this country. It was decided therefore to study these data from this standpoint.

Existing knowledge regarding the heritability of persistency and maximum yield is extremely meagre. This is particularly true of persistency. Although it is the general experience of breeders that individual cows differ in their ability to maintain milk yield, and that animals of the beef type have a very much shorter lactation than those of the

* The observed variance of (σ^2) for any character may in fact be composed of three fractions: (i) that due to hereditary differences σ_H^2; (ii) that due to environmental differences, σ_E^2; and (iii) that due to the combined influence of heredity and environment, σ_{HE}^2. The fraction σ_H^2 may again consist of: (i) the additive genetic variance which Fisher (1) calls genetic variance and Wright (27) represents by σ_g^2; (ii) variance due to dominant deviations from the additive scheme, σ_d^2; and (iii) variance due to epistatic deviations from the additive scheme σ_I^2.

dairy type, doubts have been expressed by some workers as to whether persistency is an inherited character.

Sanders (11) reported that the variability of the 'shape figure' of different lactations of the same cow was less than that of all cows considered together, and interpreted this as evidence of the fact that persistency was dependent on the individuality of the cow. He subsequently confirmed these findings from more extensive data (20). Sanders's interpretation is open to the objection that the resemblance between the different lactations of the same cow may have been due to the effect of the common environment.

Gaines (24), from a study of records of Guernsey and Holstein cows, concluded that 'persistency of lactation is nearly as definite an individual character as is initial rate of yield, judged by the fact that with the herd constant the correlation between two lactations records of the same cow $r = 0{\cdot}370$ for persistency and $r = 0{\cdot}400$ for initial rate'. However, this view was not substantiated by the results of correlations between animals related by a common line of descent. These anomalous results led Gaines to doubt if 'there is really any genetic difference between individuals within either of the two breeds with respect to persistency of production', and he went on to say that 'as compared with initial rate of yield, or rate of yield at later stages of lactation, persistency is a much less important factor in determining the ordinary lactation yield'.

Again, in another paper, Gaines (28) reported 'the stability or permanency of the persistency character is very low, the correlation between first and second records in different herds being $0{\cdot}208 \pm 0{\cdot}031$. *Persistency, as such, seems therefore to be a questionable character.* Primary rate is evidently much more definite, the corresponding correlation being $0{\cdot}420 \pm 0{\cdot}027$. The 10-months' yield, from these two, primary rate and persistency, shows still more stability $r = 0{\cdot}565 \pm 0{\cdot}022$. *From these observations the conclusion might be drawn that the lactation yield is a better measure of milking capacity than either of the lactation curve constants. Before such a conclusion is drawn, however, it is well that the inheritance of these characters be investigated.*'

Pontecorvo (9), from a study of persistency in a herd of Ayrshire cows, concluded 'that persistency is to a great extent an expression of an animal's individuality, and that the physiological causes determining the repeatability of persistency in the different lactations of the same cow are approximately of the same importance as those which tend to diversify it from one lactation to another'. In the limited data examined, no correlation was found between the persistency of dam and daughter ($r = 0{\cdot}166 \pm 0{\cdot}17$).

It is clear from the foregoing summary of previous work that the views regarding the heritability of persistency are conflicting. In reality, however, the controversy is an idle one, for strictly speaking every character is hereditary in the sense that, however suitable the environment, it cannot develop without the presence of the necessary genes. On the other hand, the genes cannot show their proper effect in the absence of a suitable environment. The point at issue really is, therefore, not whether persistency is a hereditary character, but (as with milk yield) *how far the observed differences in a particular population are hereditary and how far they are environmental.*

It appears, therefore, that two major points require to be investigated: (i) the relative stability of the different constants of the lactation curve, and (ii) the portion of the total variance of each constant which is due to hereditary causes. These points are dealt with in the succeeding sections.

1. STABILITY OF THE LACTATION CURVE

The first point worth examining is the variation of the different constants of the lactation curve in the different years and herds. A study may then be made of the intra-herd differences to determine how far they were due to differences 'between cows' as distinct from differences 'within cows'. These latter results will give the requisite information regarding the stability of the different constants in these data, as these will show to what extent a cow's performance is more apt to repeat itself in different lactations than it is likely to resemble that of its other herd-mates chosen at random.

It will be recalled that the data for the present study was derived from six herds, five being of the 'milk' type and one of the 'vessel' type. Since the conditions of management vary between these two types of herd, only the results from the five 'milk' herds are dealt with here.

(*a*) *Differences between years*

Analysis of variance of all three constants between herds and years showed a significant effect of herds and years and also significant herd-year interactions for all the constants. An analysis of variance between years for each herd separately showed that the contribution of yearly variation varied widely for the different herds as shown in Table 16.

This 'between year' effect may be the result of genetic causes, environmental causes or both. The phenotypes of the cows constituting the herds may change as a result of selection or through changes in management. It has been proposed by von Patow (29) to use the 'byre average' to allow for the influence of such variations. However, as Lush (30) has pointed out, this entirely ignores any genetic change which may be taking place.

Table 16. *The percentage of the total variance due to variation between years*

Herd	Persistency	Maximum yield	Total yield
A	3·23	56·50	57·02
B	7·89	22·72	10·78
C	3·31	6·40	2·67
D	11·05	4·62	9·97
E	11·14	16·72	18·84

(*b*) *Differences between herds*

Analysis of variance, between herds only, showed that very small proportions of the total variance were due to herd differences. The actual figures were: persistency 1·02%, maximum yield 5·82%, and total yield 4·70%.

These herds constitute, therefore, a fairly homogeneous population in contrast to those studied in Iowa by Plum (31), who found that 33% of the variance was due to herd differences. The animals in this study, however, were all of one breed in one locality, whose owners worked in close co-operation with each other.

(*c*) *Differences between and within cows*

Within each herd an analysis of variance was carried out between cows which showed the final breakdown of the variation. It was found that the proportion of the variation within herds which was due to differences between cows was 42·68% for persistency, 49·96% for maximum yield and 55·91% for total yield.

If all the differences between mature cows were genetic, apart from variations within

cows, then these would give the proportions of the variance due to genetic causes, i.e. the heritability. However, as a result of the variation between cows due to rearing and so on, the above figures are only an upper limit to the heritability. Total yield is apparently more stable during the lifetime of a cow than either maximum yield or persistency, as Gaines (28) has earlier reported. This is probably due in part to the negative 'within cows' correlation between maximum and persistency observed in the previous section. The results obtained for persistency definitely disprove Gaines's hypothesis that persistency is a questionable character.

2. METHODS OF MEASURING THE HERITABILITY OF INDIVIDUAL DIFFERENCES

(*a*) *General discussion*

The relative importance of heredity and environment in determining the observed differences between the different individuals of a population may be best studied experimentally. It is well known that no genetic differences exist among the individuals constituting a pure line, all variation in them being of the random fluctuating type and thus environmental. This was first shown experimentally by Johannson in his studies of the Princess bean (Babcock & Clausen (32)). The observed variance in a mixed population, on the other hand, may be due to both environmental and genetic differences. If, therefore, the variance observed in a mixed population is compared with that present within pure lines, an estimate can be made of the part of the total variance which is due to genetic differences.

Another experimental method of approach is to practise selection, both in the plus and minus direction, for a number of generations. The difference produced in this way between the means of the population divided by the extent of selection practised gives a measure of the true genetic variance present in that population. This result is obvious, since, as already pointed out, the resultant shift in the value of the mean due to selection is σ_g^2 (H^2 in the narrow sense) of the selection differential.

Both the above experimental methods are of limited application with such slow-breeding animals as dairy cattle. The alternative statistical method of determining the heritability from the observed value of correlation between the individuals related due to a common line of descent, though not as accurate a method, is the only practicable one.

Wright (26) has expressed the correlation between parents and offspring by the equation

$$r_{po} = \tfrac{1}{2}h^2,$$

under conditions of random mating with no dominance and no correlation between heredity and environment. It is, therefore, possible to determine that part of the variance which is due to hereditary causes by the use of this formula on the observed values of the parent-offspring correlations, provided the necessary conditions for the application of the formula are satisfied. The observed value of correlation may be too high for one or both of two reasons: (i) the breeding, instead of being random, may be assortative, and (ii) there may exist a correlation between the related individuals due to a common environment.

As Lush (33) has pointed out, when heritability is being estimated from the resemblance between relatives, the most dependable estimates are based upon the closest relationships, because the sampling errors are thereby kept relatively small. For this reason relatives more remote than half-sibs are rarely of use for estimating heritability. Further (in Lush's own words), 'the correlation between parent and offspring is generally the most useful

approach, if environmental correlations can be discounted adequately. The correlation between dam and offspring, within groups of offspring all by the same sire, is a very useful way of automatically discounting most environmental correlations and deviations from random mating.'

(*b*) *Measure of inherent milking capacity employed*

As has been pointed out already, each unselected record of a cow is a measure of her phenotype as modified by environment. No one record is perfectly correlated with another record or with the average of a number of other records, so that no one lactation is a perfect measure of a cow's phenotype. In general, the environmental conditions are as likely to be favourable as unfavourable in any lactation. If the highest corrected record is chosen to represent a cow's milking capacity, one is selecting a record made under the most favourable conditions of environment. When cows, all of which have not completed the same number of lactations, are compared on the basis of their highest corrected record, the animals with a large number of known lactations obtain an advantage over those with fewer lactations, inasmuch as they have had a greater chance to make a high record.

In the writer's opinion the procedures which are least liable to criticism are either (i) to use the record for a stipulated age for all animals regardless of the magnitude of that record, if otherwise normal, (ii) to use the average of all available, normal age-corrected records, or (iii) to use the average of the first five lactations for all cows. Since one record is more liable to be influenced by the vagaries of environment, and since method (iii) gave a small and selected sample, it was considered preferable to use the average of all available normal age-corrected records. Accordingly, the values noted in the following sections for persistency, maximum yield and total yield represent the mean values for all recorded lactations.

(*c*) *Correlation between dam and daughter*

The correlation between dam and daughter was studied within groups of daughters all by the same bull for those bulls with at least five recorded daughters. This gave 238 daughter-dam pairs from twenty-five different bulls. In most cases, records of daughter and dam were from the same herd, and nearly all the daughters of a given bull were from the same herd. The intra-sire daughter-dam correlations for persistency, maximum yield and total yield were 0·237, 0·223 and 0·263 respectively. These cannot be converted directly to estimates of heritability, as the correlation is between the averages of all the records of the animals concerned, but an estimate can be made of the heritability on the assumption that all the animals had three records. The heritability estimated in that way was found to be 29·2% for persistency, 29·8% for maximum yield and 37·2% for total yield. These figures might be a little high if there was a correlation between the environments of daughter and dam.

Another possible method of obtaining the heritability is to determine the intra-sire correlation of half-sisters. The heritability should then be four times the figure thus obtained. Such an analysis was performed on the daughters used in the daughter-dam correlation which gave the following results, the correlation being calculated within herds:

Character	Intra-sire correlations
Persistency	0·103
Maximum	0·241
Total yield	0·236

The estimates of heritability obtained from these figures would then again refer to the average of several lactations and must be compared with the daughter-dam correlations rather than with the corrected estimates given above. It will be seen that the intra-class correlation is much higher than expected in the case of maximum and total yield. This is presumably due to high environmental correlations between half-sisters. The high year-to-year variations previously found would account for a high proportion of this. A separate analysis for each herd shows that those herds showing high yearly differences are precisely those showing high family differences, so that these two effects are entangled with each other.

SUMMARY

1. A study has been made of the interrelationships between persistency and maximum yield as components of the lactation curve, and of the importance of environment and heredity in determining the shape of the lactation curve of Ayrshire cows.

2. Persistency was defined as the linear constant of the exponential curve expressed as a percentage, *maximum yield* as the highest 30-day yield as calculated from the records, and *total yield* as the yield of milk from 15 days to 8½ months after calving.

3. The study was based on milk records computed by the official method of the Scottish Milk Records Association. The data used in much of the work was obtained from five herds of Ayrshire cows and involved 2392 lactations during the period 1920–39.

4. In an exploratory test involving 100 lactations it was found that the exponential curve fitted ninety-five of the lactation curves and that the fit was improved in forty-eight of these ninety-five curves by using the parabolic exponential curve. Five of the lactations were too erratic to be fitted by any simple mathematical curve.

5. Monthly variations accounted for 9·57, 1·05 and 0·73% of the total variation for persistency, maximum yield and total yield respectively. Correction factors were calculated for all three constants.

6. Differences in age accounted for 17·78, 31·16 and 14·67% of the total variation in persistency, maximum yield and total yield. Factors showing the value of each lactation as a percentage of the fourth lactation were calculated by the 'paired-lactation' method and by the 'lumped-lactation' method and the effect of selection on the latter method demonstrated. By the 'paired-lactation' method it was shown that persistency declined with age from the first lactation, that maximum yield rose to a peak at the fourth lactation after which it declined, and that total yield showed a peak at the fifth lactation and then declined.

7. The average correlations between lactations were +0·49, +0·65 and +0·70 for persistency, maximum yield and total yield. In predicting the current yield there was little to be gained by basing the prediction on more lactations than the immediately preceding one.

8. Significant within cow correlations were shown between persistency and maximum yield ($r = -0{\cdot}17$), persistency and total yield ($r = +0{\cdot}37$), and between maximum and total yield ($r = +0{\cdot}70$). The multiple correlation (R) of maximum yield and persistency with total yield was +0·854 within cows showing that these two factors accounted for most of the variation in total yield. Maximum yield was shown to be approximately three times as important as persistency.

9. Analyses of variance showed significant differences in persistency, maximum yield and total yield between herds but these differences accounted for only 1 to 6% of the

variation. Differences between years were also significant and those in herd-year interaction accounted for up to 11, 56 and 57% of the variation for persistency, maximum yield and total yield respectively. Within herds, differences between cows accounted for 43, 50 and 56% of the variation for the same three constants. The high value for persistency is emphasized.

10. Using the means of all age corrected records the heritability of persistency, maximum yield and total yield was estimated from within-sire dam-daughter correlations to be 29·2, 29·8 and 37·2%. These figures were perhaps slightly biased by correlation in the environments of dam and daughter, so that the true values might be lower.

REFERENCES

(1) Fisher, R. A. (1930). *The Genetical Theory of Natural Selection.* Oxford: Clarendon Press.
(2) Sturtevant, A. H. (1886). *5th Ann. Rep., N.Y. St. agric. Exp. Sta.* pp. 21–3.
(3) Brody, S., Ragsdale, A. C. & Turner, C. W. (1922). *J. gen. Physiol.* **5**, 441–4.
(4) Turner, C. W., Brody, S. & Ragsdale, A. C. (1923). *J. Dairy Sci.* **6**, 527–31.
(5) Johannsen, K. I. (1939). Private communication to the author.
(6) Fisher, R. A. (1938). *Statistical Methods for Research Workers*, 7th ed. pp. 27–30. Edinburgh: Oliver and Boyd.
(7) Fisher, R. A. (1948). *Statistical Methods for Research Workers*, 10th ed. pp. 24–9. Edinburgh: Oliver and Boyd.
(8) Brody, S. (1927). *Res. Bull. Mo. agric. Exp. Sta.* no. 97, p. 70.
(9) Pontecorvo, G. (1940). *J. Dairy Res.* **11**, 113–20.
(10) Gavin, W. F. (1913). *J. agric. Sci.* **5**, 309–19.
(11) Sanders, H. G. (1923). *J. agric. Sci.* **13**, 169–79.
(12) Sanders, H. G. (1927). *J. agric. Sci.* **17**, 502–23.
(13) Gaines, W. L. (1926). *J. gen. Physiol.* **9**, 325–32.
(14) Østergaard, P. S (1931). *9th International Dairy Congress*, pp. 49–59.
(15) Kartha, K. P. R. (1934). *Indian J. vet. Sci.* **4**, 124–47.
(16) Turner, C. W. (1939). Private communication.
(17) Robb, R. A. (1940). Private communication.
(18) Watson, S. J. (1939). *The Science and Practice of Conservation: Grass and Forage Crops*, **1**. London: The Fertilizer and Feeding Stuffs Journal.
(19) Turner, C. W. (1927). *J. Dairy Sci.* **10**, 95–105.
(20) Sanders, H. G. (1930). *J. agric. Sci.* **20**, 145–85.
(21) Tocher, F. (1928). *Biometrika*, 20B, 105–244.
(22) Kay, R. R. & McCandlish, A. C. (1929). *J. agric. Sci.* **19**, 342–72.
(23) Glen, J. & McCandlish, A. C. (1930). *J. agric. Sci.* **20**, 45–52.
(24) Gaines, W. L. (1927). *Bull. Ill. agric. Exp. Sta.* no. 288.
(25) Gooch, M. (1935). *J. agric. Sci.* **25**, 71–102.
(26) Wright, S. (1921). *Genetics*, **6**, 111–78.
(27) Wright, S. (1920). *Proc. nat. Acad. Sci., Wash.*, **6**, 320–32.
(28) Gaines, W. L. (1931). *9th International Dairy Congress*, pp. 140–9.
(29) von Patow, C. (1930). *Z. Zucht.* B, 17, 3–159.
(30) Lush, J. L. (1936). *J. Hered.* **27**, 201–3.
(31) Plum, M. (1933). *Proc. Amer. Soc. Anim. Prod.* pp. 53–7.
(32) Babcock, E. B. & Clausen, R. E. (1927). *Genetics in Relation to Agriculture.* New York and London: McGraw-Hill Book Company Inc.
(33) Lush, J. L. (1939). *Methods of Measuring the Heritability of Individual Differences among Farm Animals*, pp. 34–6. Paper read at the International Genetics Congress, Edinburgh, Section D.

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425. FURTHER INVESTIGATIONS ON THE PRESENCE IN RAW MILK OF A BACTERICIDAL SUBSTANCE SPECIFIC FOR CERTAIN STRAINS OF COLIFORM ORGANISMS

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(With 2 Figures)

INTRODUCTION

Since the publication of the original paper (1) on the occurrence of slow-reducing coliform organisms in raw milk, further investigational work has been undertaken. During the course of the work two different types of coliform organisms appeared to be responsible for the slow reduction of methylene blue: (*a*) those having a long lag phase of growth, and (*b*) those destroyed by a bactericidal substance present in raw milk.

This was demonstrated by inoculating raw 'sterile'* milk with approximately 200,000 coliform organisms per ml., previously isolated in pure culture from designated milk having a long methylene-blue reduction time, but containing coliform organisms in $\frac{1}{100}$ ml., and carrying out the following tests:

(*a*) Methylene-blue test at the time of inoculation.

(*b*) Colony count on MacConkey agar at the time of inoculation.

(*c*) Colony count on MacConkey agar after 6 hr. incubation at 37° C.

Of 212 cultures tested, fifteen did not reduce methylene blue in 6 hr. or show any appreciable increase in colony count, indicating that the slow reduction of methylene blue was due to a long lag phase of growth; seven other cultures did not reduce methylene blue in 6 hr., but showed a marked decrease in colony count, indicating that the slow reduction of methylene blue was due to the destruction of the coliform organisms by some factor which will be referred to throughout the paper as a bactericidal substance. The remaining cultures were normal types reducing methylene blue in less than 6 hr. and showing a marked increase in colony count.

TYPING OF COLIFORM ORGANISMS SUSCEPTIBLE TO THE BACTERICIDAL SUBSTANCE

The seven cultures susceptible to the bactericidal substance gave negative results with the Eijkman test, proving that the organisms were non-faecal.

A further thirteen susceptible cultures were isolated from various sources, and these thirteen, together with the seven previous susceptible cultures, were submitted to full differential tests, the results of which are set out in Table 1.

Table 1 shows that the bactericidal substance present in raw milk was not specific for any particular type of coliform organism; but it is of interest that, up to date, no *Bact. coli* type I (2) has been found to be susceptible to the bactericidal substance.

* Milk from a normal cow taken with aseptic precautions.

Table 1. *Differential tests on the coliform organisms susceptible to the bactericidal substance present in milk*

Culture No.	Koser's citrate	Indol 3 days	Gelatin	Eijkman test at 44° C.	Voges-Proskauer (days) 1	2	3	4	5	6	7	Methyl red	Type
1	−	−	−	−	−	−	−	−	−	−	−	+	*Bact. coli* type II
2	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
3	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
4	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
5	+	−	−	−	+	−	−	−	−	−	−	+	Irregular strain
6	+	+	−	−	−	−	−	−	−	−	−	+	Intermediate type II
7	+	−	−	−	+	+	+	+	+	+	+	−	*Aerogenes* type I
8	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
9	+	−	−	−	+	+	+	+	+	+	+	−	*Aerogenes* type I
10	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
11	−	−	−	−	−	−	−	−	−	−	−	+	*Bact. coli* type II
12	+	−	−	−	+	+	−	−	−	−	−	+	Irregular strain
13	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
14	+	−	−	−	+	+	+	+	+	+	+	−	*Aerogenes* type I
15	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
16	+	−	−	−	+	+	−	−	−	−	−	+	Irregular strain
17	+	−	−	−	+	+	+	+	+	+	+	−	*Aerogenes* type I
18	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I
19	+	−	−	−	+	+	+	+	+	+	+	−	*Aerogenes* type I
20	+	−	−	−	−	−	−	−	−	−	−	+	Intermediate type I

INVESTIGATIONS ON THE MODE OF ACTION AND NATURE OF THE BACTERICIDAL SUBSTANCE

(1) *Effect of Heat*

(*a*) *Temperature of destruction of the substance*

It was previously reported that the bactericidal substance was destroyed at 70° C. for 1 hr. in the same way as Hobbs's growth-inhibitory factor (3). To find whether Hobbs's factor was similar to the bactericidal substance, raw milk was heated for various times and temperatures before inoculation with a culture of the susceptible organism.

The temperatures used were 70° C. for 1 hr., 63, 55 and 45° C. for 30 min. The bactericidal substance was completely destroyed when raw 'sterile' milk was heated to 55° C. for ½ hr. Susceptible organisms when inoculated into this milk were not destroyed, but reduced methylene blue in less than 6 hr. When the milk was heated to 45° C. for ½ hr. the substance remained active, and susceptible organisms, when inoculated into this milk, showed a 99% decrease, and methylene blue was not reduced in 6 hr. The substance is therefore destroyed at a point between 45 and 55° C. for ½ hr. The exact temperature of destruction of the substance was then determined by heating milk for ½ hr. to various temperatures between 45 and 55° C. The results proved the temperature of destruction to be very critical, i.e. at 52° C. the substance was not destroyed, whereas at 53° C. the substance was completely destroyed.

(*b*) *Optimum temperature for the bactericidal action of the substance*

Raw milk inoculated with 200,000 organisms per ml. was held at 4, 18, 23, 26, 29, 37 and 45° C. for 4 hr. to determine the optimum temperature for the bactericidal action of the substance. Fig. 1 shows that at 4 hr. the substance reaches its maximum activity when the milk is held at 29, 37 and 45° C. Below 29° C. the action of the substance is much slower, and 23° C. the substance does not have any lethal effect. This, however, was

a temperature effect rather than inactivity of the substance, because when milk was held for 9 hr. at these lower temperatures, the activity of the substance increased in proportion to the temperature (Fig. 2).

An attempt was made to define the activity of the substance above 45° C., but the thermal death-point of the susceptible organisms was so low (i.e. 55° C. for 10 min. in physiological saline) that it was not certain whether the destruction of the organisms at these temperatures was due to the substance in the milk or to the death of the organisms by heat. This low thermal death-point was common to all organisms susceptible to the bactericidal substance.

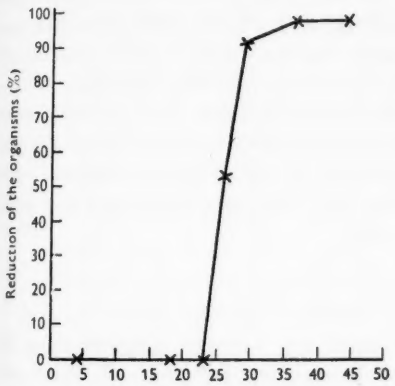

Fig. 1. Temperature (° C.) at which milk was held for 4 hr.

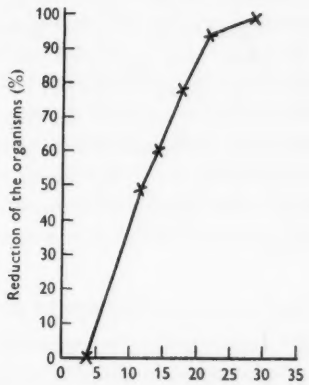

Fig. 2. Temperature (° C.) at which milk was held for 9 hr.

Figs. 1, 2. Effect of temperature on the activity of the bactericidal substance present in raw milk.

(2) *Presence of the bactericidal substance in whey*

Whey prepared from raw 'sterile' milk appeared to contain the bactericidal substance in about the same amount as raw milk, which suggests that the substance is not removed with the curd in the preparation of the whey. This limited the possibility of isolation of the substance in a chemically pure form to those fractions present in whey. Attempts to isolate the substance have so far been unsuccessful, and attention has therefore been given to the action of the substance on the organisms when grown in whey. Continuous direct microscopical examination was made of a suspension of living organisms in raw 'sterile' whey, on a warm stage at 37° C. The organisms immediately after inoculation were clearly visible and motile, but motility soon ceased and the organisms gradually became ill-defined. This phenomenon was not due to visible agglutination or lysis, but to the inability of the organisms to reflect light. This suggests that the surface of the organism was altered in some way and rendered incapable of reflecting light. This phenomenon coincided with the death of the organisms as determined by the colony count.

(3) *Presence of the bactericidal substance in cows' blood serum*

The presence of the substance in whey suggested that it might also be found in cows' blood serum. Some samples of cows' blood serum were added to physiological saline to give a 1% concentration of serum. These solutions were then inoculated with 200,000

susceptible organisms per ml., and incubated at 37° C. for 4 hr. Colony counts at 4 hr. showed decreases of 99% of the counts at the time of inoculation. A bactericidal substance is therefore present in serum at a concentration at least equal to that of the substance in raw milk. As 1% of serum in physiological saline gave a decrease of 99% in the numbers of organisms after incubation at 37° C. for 4 hr., it was necessary to determine the minimum concentration of serum to cause this reduction. Varying concentrations of blood serum in physiological saline, ranging from 0·1 to 1%, were tested. The minimum concentration necessary to effect 99% reduction was found to vary with the age of the serum, 0·25% giving 99% reduction with fresh serum; but at least 0·35% was required to give a 99% reduction when the serum had been aged in a refrigerator (4° C.) over a period of weeks. This suggested that serum contained much more of a lethal substance than raw milk. Hitherto only undiluted raw milk had been used for estimating the percentage destruction of the organisms, but less than 100% may have given 99% reduction. Therefore, dilutions of a raw milk, in milk that had been heated to 55° C. for ½ hr. to destroy the substance, were tested to ascertain at which dilution 99% reduction after 4 hr. at 37° C. was obtained. It was found that 20% raw milk in heated milk was necessary to give 99% reduction for this sample. It is evident that this particular serum contained very much more of a lethal substance than the milk.

(4) *Presence of the substance in other blood sera*

At the same time as the cows' blood was obtained, sera were also prepared from the blood of the following animals: sheep (male and female), pig, bull and calf. These sera were then tested for the presence of bactericidal substances.

It was not possible to take these blood samples under sterile conditions, and since sterility was necessary for the tests the sera were Seitz filtered, after it had been proved that they remained active after filtration.

As with cows' serum, 1% concentrations were prepared in physiological saline, inoculated with 200,000 susceptible organisms per ml. and incubated at 37° C. for 4 hr. Colony counts at 4 hr. in each case showed a 99% reduction in the numbers of organisms at the time of inoculation, thus proving the presence of a lethal substance in the sera of several animals other than the cow.

The substance present in cows' serum was inactivated in the same way as the substance present in milk by heating to 55° C. for ½ hr. The addition of 1% raw serum to heated milk always replaced the substance destroyed by heating. The substances in blood and serum may not be identical but they react similarly.

(5) *Variations in the amount of substance present in raw milk*

During the course of the investigations test cultures occasionally appeared to lose their susceptibility to the bactericidal substance; at first this phenomenon was attributed to the culture, but later, by testing it simultaneously in different milks, it was found to be due to the variations in the amount of substance present in different raw milks.

The greatest variations were obtained when testing goats' milk for the presence of bactericidal substance; sometimes the culture inoculated into the milk and held for 4 hr. at 37° C. was destroyed, and sometimes was virtually unaffected, depending apparently on the amount of substance present in the goats' milk.

(6) *Quantitative estimation of the bactericidal substance present in raw cows' milk*

Having found that the amounts of substance in goats' milk varied, attempts were made to estimate quantitatively the amount of substance present in normal cows' milk, and the following methods were investigated:

(*a*) *Inoculation of large numbers of organisms*

All previous experiments had been carried out with an inoculum of approximately 200,000 susceptible organisms per ml. Usually a reduction of 99% in numbers of organisms after incubation for 4 hr. at 37° C. was found. The possibility was considered that heavy inoculations might absorb the substance in proportion to the numbers of organisms inoculated, and so form a basis for quantitative estimation.

Varying inoculations, up to 200,000,000 per ml., were made with 'sterile' raw milk and incubated at 37° C. for 4 hr., plate counts being carried out at inoculation and after incubation for 4 hr. In each case, even with the very heavy inoculations, there was always approximately 99% reduction in numbers of organisms. It was not therefore possible to adopt this method to estimate the amount of substance present in raw cows' milk.

(*b*) *Repeated inoculations into milk held at* 37° C.

As single heavy inoculations were 99% destroyed it was thought that perhaps repeated inoculations with a smaller number of organisms might give better results. Raw milk inoculated with 13,000,000 susceptible organisms per ml. was held at 37° C., and at 2-hourly intervals other additions of approximately the same number of organisms was made to the same milk. At each fresh inoculation a portion of the milk was withdrawn to act as a control. At the end of the experiment therefore there was one sample of milk with one inoculation, another with two inoculations and so on.

Colony counts were made on all samples at 2-hourly intervals; thus, for example (Table 2), the following four colony counts were carried out simultaneously and are comparable: colony count at 8 hr. on milk inoculated once, colony count at 6 hr. on milk

Table 2. *Inactivation of the bactericidal substance by living organisms inoculated into raw 'sterile' milk at intervals of 2 hr.*

Milk with 1 inoculation:	Colony counts per ml.
At inoculation	13,000,000
After 2 hr. at 37° C.	1,700,000
After 4 hr. at 37° C.	100,000
After 6 hr. at 37° C.	200,000
After 8 hr. at 37° C.	100,000
Milk with 2 inoculations:	
At inoculation	15,000,000
After 2 hr. at 37° C.	1,400,000
After 4 hr. at 37° C.	200,000
After 6 hr. at 37° C.	2,200,000
Milk with 3 inoculations:	
At inoculation	12,000,000
After 2 hr. at 37° C.	8,900,000
After 4 hr. at 37° C.	21,000,000
Milk with 4 inoculations:	
At inoculation	20,700,000
After 2 hr. at 37° C.	34,800,000

inoculated twice, colony count at 4 hr. on milk inoculated three times, and colony count at 2 hr. on milk inoculated four times.

Table 2 shows that it was impossible to obtain accurate estimations of the destruction of organisms by the substance because of proliferation of organisms from previous inoculations, i.e. milk that had four inoculations showed an apparent increase in numbers of organisms after 2 hr., but the actual count was only 13,000,000 per ml., because 21,000,000 of these organisms were present at the 4 hr. period in the milk that had had three inoculations. This method was, therefore, unsatisfactory for quantitative estimation of the substance present in milk because of the corrections that had to be made and the subsequent errors that might arise.

(*c*) *The addition and subsequent removal of living susceptible organisms*

Repeated inoculations with living organisms could not be used for the estimation of the amount of bactericidal substance present in milk, and a method was therefore adopted in which the organisms were suspended in milk for a period of time and then removed by Seitz filtration before again inoculating with susceptible organisms. It was found to be impracticable to pass sufficient milk through a Seitz filter owing to the slowness of filtration, and whey, known to contain the substance, was therefore used. Whey was prepared from raw 'sterile' milk and inoculated with 20,000,000 susceptible organisms per ml. and incubated for 2 hr. at 37° C. This was then Seitz filtered and the filtrate reinoculated with 200,000 living susceptible organisms per ml. and incubated at 37° C. for 4 hr. Colony counts at 4 hr. always showed an increase over the initial counts, indicating the absence of the bactericidal substance at the time of reinoculation. This proved that the substance could be removed from whey by the addition of living organisms subsequently removed by filtration. Filtration was, however, still slow, and it is likely that there was some loss of the substance by adsorption to the filter. Absorption by the addition of dead organisms was, therefore, attempted.

(*d*) *The addition of dead susceptible organisms*

An 18 hr. old susceptible culture was washed from a slope with sterile physiological saline, adjusted to an opacity of no. 6 Brown's tube, and laboratory pasteurized at 63° C. for $\frac{1}{2}$ hr. to kill the organisms. An inoculation of 1 ml. of the pasteurized suspension was then made into 100 ml. of raw 'sterile' milk to give an approximate number of 20,000,000 organisms per ml., and incubated at 37° C. for 2 hr. before reinoculations with 200,000 living susceptible organisms per ml. Colony counts carried out at the time of reinoculation and after 4 hr. at 37° C. showed that the substance had been partially inactivated by the dead organisms.

Repeated tests on milk from different cows, using the above technique, gave varied results, which verified the earlier statement that the amount of substance was not constant from milk to milk. To substantiate these findings, and to prove whether this technique could be adopted for the measurement of the amount of substance present in any milk, varying inoculations of dead susceptible organisms from 10 to 100 million per ml. were made into a number of samples of 'sterile' raw milk, and reinoculated with living susceptible organisms after 2 hr. at 37° C. The results were encouraging, and this technique is now being developed for the assay of different milks.

(*e*) *Variations in inactivation of the substance due to killing the organisms at different temperatures*

Since it was likely that a few cells might survive heat treatment at 63° C. for ½ hr., higher temperatures were used to kill the organisms. A saline suspension, opacity no. 6 Brown's tube, of a susceptible culture was heated (*a*) at 63° C. for ½ hr., (*b*) at 70° C. for 1 hr. and (*c*) boiled for 10 min. before inoculating into raw 'sterile' milk. The milk was incubated at 37° C. for 2 hr. and then reinoculated with living organisms.

The results showed that the rate of inactivation of the substance in milk was closely related to the temperature of destruction of the culture; the pasteurized culture inactivated most of the substance in the milk, as was shown by a slight increase in numbers of the reinoculated organisms after 4 hr. at 37° C., whereas the boiled culture did not inactivate any of the substance and the reinoculated organisms were destroyed. The apparent increase when the culture was pasteurized was not due to the survival of individual cells, as all control tests on milk inoculated with dead organisms, after incubation at 37° C. for 6 hr., were sterile, or gave only very small colony counts.

It follows, therefore, that it is important to control the temperature used to destroy the culture, otherwise larger amounts of bactericidal substance will be recorded as being present in the raw milk under test, when actually the culture had been overheated and was unable to inactivate the substance so readily.

(*f*) *Inactivation of the substance by dead non-susceptible cultures*

As a matter of interest other cultures were tested in the same way as described, to find whether other types of organisms could inactivate the bactericidal substance which was present in raw milk and which was specific for the coliform organisms dealt with in this paper. A non-susceptible strain of coliform organism of faecal origin and a strain of *Streptococcus lactis* were used in a pilot experiment, both being killed by pasteurizing at 63° C. for ½ hr. Both the *Str. lactis* and the non-susceptible *coli* were found to inactivate some of the substance, but the inactivation was less than that by the pasteurized susceptible culture.

(*g*) *The addition of varying amounts of raw milk to milk heated to* 55° C. *for* ½ *hr.*

Different percentages of raw milk were added to the heated milk and inoculated with susceptible organisms, and incubated at 37° C. for 4 hr. Colony counts were carried out at the time of inoculation and after 4 hr. at 37° C., and the percentage destruction of the organisms calculated. The percentage addition of raw milk required to give 99% destruction was used as a measure of the amount of substance present.

Wide variations in the amount of substance present in the milk of different cows were found. It was necessary in some cases to add only 20% of raw and in other cases 90% to effect 99% destruction of the susceptible organisms.

DISCUSSION

A substance specifically bactericidal for certain strains of coliform organisms is present in cows' milk. Experiments have so far been directed mainly to the establishment of a technique for the estimation of the amount of substance present in the milk of different cows.

The addition of living organisms to absorb the substance from milk proved unsuitable because of the proliferation of a few survivors on incubation.

Seitz filtration of whey, proved to contain the substance, failed, owing to the time involved.

It was found that heat-killed organisms readily absorbed the substance provided that the temperature used was not too high. Organisms heated to higher temperatures failed to absorb the substance presumably owing to the changes produced in the cells. A promising method was finally evolved. This consisted in adding to milk heated at 55° C. for ½ hr. various proportions of the raw milk to be assayed, together with the susceptible organisms. Great variations in the amount of bactericidal substance present in different raw milks were found.

Little attempt has yet been made to isolate or identify the substance responsible for the phenomena described. The fact, however, that its concentration in milk varies from cow to cow seemed of sufficient interest to warrant publication in view of the suspected variation in individual susceptibility of cows to udder infections of various kinds.

REFERENCES

(1) Morris, C. S. (1943). *J. Dairy Res.* **13**, 115.
(2) Topley & Wilson (1936). *The Principles of Bacteriology and Immunity*, 2nd ed. London: Arnold.
(3) Hobbs, B. C. (1939). *J. Dairy Res.* **10**, 35.

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637.133:576.8

426. SOME BACTERIOLOGICAL ASPECTS OF THE DETERIORATION OF PASTEURIZED MILK

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(With 3 Figures)

The Milk (Special Designations) Regulations, 1941 (1) prescribed an alternative to the Low-Temperature 'Holder' (L.T.) method of pasteurization; this High-Temperature Short-Time (H.T.S.T.) process, as it is called, has been widely adopted and is now used to a greater extent than the L.T. 'Holder' method.

The choice of the time/temperature combinations for the two processes which are permissible in England and Wales, viz. 145–150° F. for not less than 30 min. for the L.T. 'Holder' method, and not less than 161° F. for a minimum time of 15 sec. in the case of the H.T.S.T. method, is based fundamentally on the work of North & Park (2), who established certain time/temperature relationships necessary to ensure the destruction of tubercle bacilli in milk. There is very little doubt that both methods of pasteurization afford an adequate margin of safety against the survival of pathogens, but as yet there is insufficient evidence to indicate whether the bacteriological and chemical effects of the two processes are similar.

Attempts to compare the two methods of pasteurization in the laboratory and even on a commercial scale have yielded results which in the main are still rather confusing. Provan & Rowlands (3), Clegg & Lomax (4), Davis & Watson (5), McKenzie & Booth (6), McKenzie (7), and Thomas, Griffiths, Morgan & Lewis (8) have examined a large number of pasteurized milks in different areas of the country by means of the customary tests (statutory phosphatase and methylene-blue reduction tests (M.B.), colony counts at 37° C. and coliform tests) and have assessed the general efficiency of pasteurization and in some instances compared the two systems of treatment. The validity of such comparisons is open to criticism, in that many of the samples were not obtained from the heat-treatment plant itself, but were bottles of milk obtained from filling machines or from cold stores. In addition, the treatment of samples between the time of collection and examination was not always uniform.

Thomas & Clegg (9) point out that whilst such surveys may serve as a guide and indicate bacteriological differences between the two methods of pasteurization, any attempt to evaluate efficiency can be made only on plants which are cleaned and operated under standard conditions, and on which duplicate bulks of the same milk are processed.

Experiments carried out by Mattick & Hiscox (10) on a commercial plant showed reasonable agreement between the plate counts at 37° C. of milk pasteurized by the H.T.S.T. method (165° F. for 17 sec.) and laboratory L.T. 'Holder' method (145° F. for 30 min.). In another series of experiments by the same authors (10) plate counts at 30° C. were considerably lower in the case of H.T.S.T. samples (162·5° F. for varying times between 15·5 and 21·5 sec.) than in corresponding laboratory L.T. 'Holder' pasteurized samples (145° F. for 30 min.). Reid's (11) work indicated the superiority of the L.T. 'Holder' over

the H.T.S.T. process from the point of view of bactericidal efficiency. This conclusion was based on the plate counts and M.B. reduction times at 37° C. of commercial H.T.S.T. (162° F. for 15 sec.) and L.T. 'Holder' (146° F. for 30 min.) samples, which had been stored for 12 hr. at 38° F., plus a further 24 hr. at atmospheric shade temperature not exceeding 65° F.

More recent experiments carried out by Egdell & Bird (12) on a limited number of pasteurized milk samples, from both H.T.S.T. and L.T. 'Holder' plants, appeared to give equally good results.

The U.S. Public Health Service Milk Ordnance and Code (13) recommends 143° F. for 30 min. for the 'Holder' method and 160° F. for 15 sec. for the H.T.S.T. method; these treatments are claimed to ensure an adequate margin of safety against the survival of pathogens (Dahlberg (14), Holland & Dahlberg (15), Holmquist (16), Mattick & Hiscox (17)).

The comparative efficiency of the two methods of treatment has been noted by several workers, some of whom have considered other times and temperatures. The consensus of opinion favours the view that the H.T.S.T. is a less rigid form of heat treatment than the L.T. 'Holder' method as gauged by the relative numbers of surviving thermoduric organisms (Yale (18), Quinn & Burgwald (19), Workman (20), Parfitt (21), Kreuger (22), Weinrich (23), Fabian (24), Millenky & Brueckner (25)).

Hileman & Leber (26) compared the plate counts of milk plant pasteurized by the H.T.S.T. method (161° F. for 15 sec.) with those of corresponding samples pasteurized in the laboratory by L.T. 'Holder' (147° F. for 30 min.) and simulated H.T.S.T. methods (161° F. for 16 sec.). The conditions of heating and holding with the H.T.S.T. samples were widely different, and to some extent can be held to invalidate certain conclusions reached. These workers found that the number of organisms surviving the two laboratory methods of pasteurization were approximately the same when counts were made at 32° C., but at 37° C. the difference increased as the samples became more densely populated. It was also shown that fewer organisms survive the L.T. 'Holder' method. Lipman, Frandsen & Lindquist (27), however, expressed the opinion that the electropure process (162° F. for 16 sec.) and the L.T. 'Holder' (143° F. for 30 min.) process have an equivalent bactericidal effect.

Burgwald (28) and Fabian (24) assert that the bacterial counts at 37° C. of the raw and corresponding H.T.S.T. plant pasteurized milk (160° F. for 15 sec.) show no relationship to one another. On the other hand, Hileman & Leber (26) found some degree of association between the plate counts at 37° C. of raw and laboratory L.T. 'Holder' pasteurized milk samples.

The present investigation was prompted by the lack of critical and comparative bacteriological information on the sequence of changes which occur when raw bulk tank milk of commercial quality is pasteurized by the two methods permitted in England and Wales.

OUTLINE OF INVESTIGATION

The experimental work was planned to secure the following information:

(1) The numbers of organisms in selected raw milk supplies which survive the effects of heat treatment by the H.T.S.T. (162° F. for 15 sec.) and the L.T. 'Holder' (145° F. for 30 min.) methods of pasteurization.

(2) An estimation of the increase in numbers of organisms, at daily intervals, on ageing the two series of pasteurized milk samples at 18° C.

(3) An estimation of the keeping quality (K.Q.) of the pasteurized milk samples aged at 18° C., as given by methylene-blue and resazurin reduction tests, alcohol precipitation and clot-on-boiling (C.O.B.) tests.

(4) The interrelationships, if any, between the numbers of bacteria present in the raw, fresh pasteurized and aged pasteurized milk samples and other test results.

(5) The work outlined above was repeated, but using a large bulk (3000 gal.) of raw milk which had been aged at 40–50° F. (4·4–10° C.) for 24 hr. prior to pasteurization.

The process of deterioration of both the fresh and aged raw milk and the corresponding pasteurized milk samples (L.T. 'Holder' and H.T.S.T.) was further studied by examination of the bacterial floras; and an attempt has been made to elucidate the processes of deterioration which took place. The possibilities of relationships between the floras of the fresh and aged raw milks and the corresponding pasteurized milks were also examined. The pasteurized milk samples were aged at 18° C.

Scrupulous care was taken to avoid post-pasteurization contamination of samples, since one of the main objects of the experiments was to make a critical comparison of the effects of the two heating processes. Consequently, the experimental results obtained may not be applicable to some pasteurized milk which is produced commercially in which the effects of contamination are often marked.

EXPERIMENTAL METHODS

(1) *Sampling methods*

Owing to difficulties in reproducing the H.T.S.T. method of pasteurization accurately on a laboratory scale, and in such manner that the required amounts of the pasteurized samples (approximately 1500–1600 ml.) were available for experimental purposes, a standard commercial H.T.S.T. plant (comprising normal heat exchanger with box type 'Holder') was used.

Using a special technique to suit the circumstances (described later), the corresponding raw milk was sampled from the raw-milk feed tank to the machine. The rate of raw milk sampling was adjusted to give approximately twice the volume of the H.T.S.T. sample, in order that an equal amount could be pasteurized in the laboratory by the L.T. 'Holder' method. Three approximately equal milk samples (each of 1500–1600 ml.) of raw milk, L.T. 'Holder' pasteurized milk, and H.T.S.T. pasteurized milk were therefore obtained for each experiment.

The plant used for experimental purposes was of 1000 gal. per hr. capacity, the calculated details of the time/temperature conditions prevailing in the milk during its passage through the plant being represented in Fig. 1. Slight differences from the calculated times required to heat the milk to 162° F. and the time of 'holding' at the same temperature were noted when the dye test elaborated by Dummett & Mongar (29) was carried out on water subjected to the same process. The summarized results of these tests, which were carried out at monthly intervals, on the day preceding sampling are as follows:

	Calculated (sec.)	Dummett & Mongar test (41 tests) (sec.)
Time to pass through regenerator (heating) and hot-water heating sections	49·04	48·0–51·0
Time to pass through 'Holder' plate	15·50	15·0–16·0

Prior to sampling, the plant was cleaned and sterilized, the latter process consisting of circulating hot water at a temperature of 195–205° F. (90·6–96·1° C.) for 45 min. At the end of this period the raw milk cock to the feed tank of the machine was opened, and simultaneously the water flow from the cooler section was diverted to waste. This method of beginning the 'run' avoided a flow diversion of the milk and ensured that the pasteurized milk was only heated once. As soon as the master thermometer adjacent to the sampling cock indicated a temperature of $162 \pm 0{\cdot}2°$ F. samples were taken as follows:

(*a*) *Raw milk*

Raw milk samples were obtained from the float-controlled feed tank to the pasteurizing plant by means of a sterile $\frac{1}{2}$ in. diameter glass syphon. Before sampling commenced the rate of flow was adjusted to approximately 600 ml. per min. by means of a screw clip on the rubber tubing connecting the syphon and delivery nozzle. 3000–3500 ml. of raw milk were collected in a sterile Erlenmeyer flask observing the usual aseptic precautions.

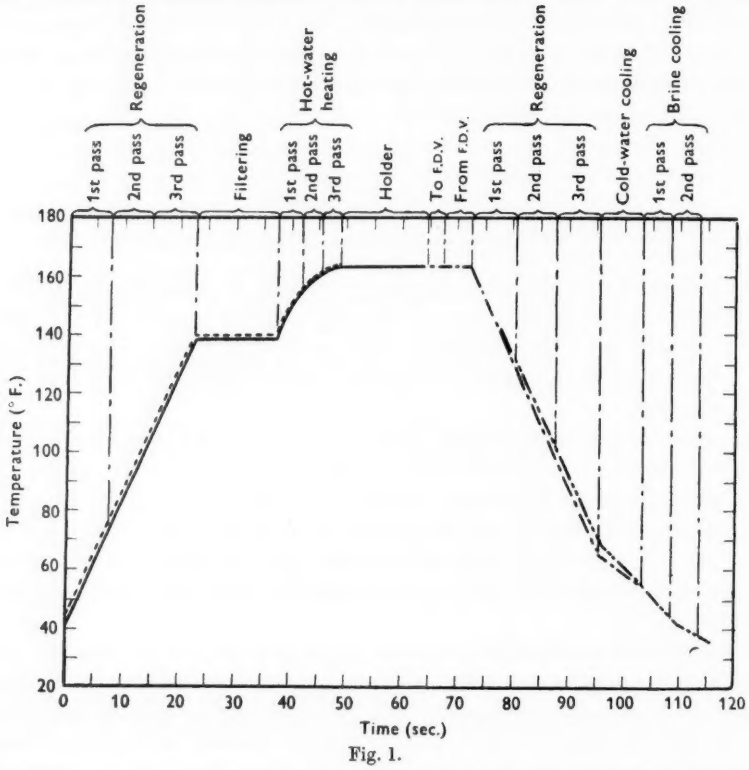

Fig. 1.

Whilst sampling was in progress, the raw milk flow into the storage tank was shut off, and hence the samples collected could be considered representative of the contents of the storage tank.

(*b*) *High-temperature short-time pasteurized milk*

As soon as the pasteurizing plant was operating satisfactorily, the delivery nozzle of the small sampling cock on the delivery pipe from the 'Holder' plate was flamed and the tap

fully opened. An interval of 64–65 sec. was allowed to elapse between sampling of the raw and the H.T.S.T. pasteurized milk, to allow for the time taken for the raw milk to pass from the float-controlled feed tank through the plant as far as the special sampling cock (Fig. 2). The sample was collected in a sterile 5000 ml. Erlenmeyer flask which was immersed to the neck in cold calcium chloride solution. To facilitate cooling the hot milk was allowed to flow down the inclined side of the flask, the latter being rotated slowly whilst sampling proceeded. The $CaCl_2$ solution was maintained within temperature limits of 20–30° F. (−6·7 to −1·1° C.) to ensure immediate cooling of the milk to a temperature

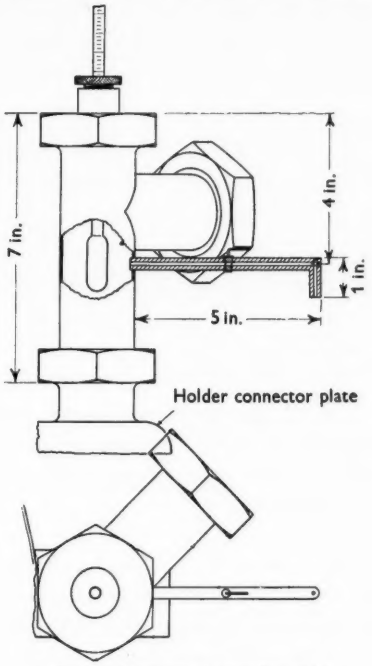

Fig. 2. Diagram of sampling cock.

Fig. 3.

not exceeding 70° F. (21·1° C.). Further cooling to approximately 45° F. (7·2° C.) was effected by holding the flask and contents in the brine solution. When cooled, samples were transferred to the refrigerator until required.

(c) *Low-temperature 'Holder' pasteurized milk*

The L.T. 'Holder' pasteurized milk samples were prepared in the laboratory from the raw milk samples obtained from the float-controlled feed tank. The flask containing the sample was first shaken for 3 min., then half the contents transferred to a sterile 2500 ml. conical flask. Forty-three sterile 6 × 1 in. thin-walled test-tubes were each filled with 40 ml. of the raw milk by means of sterile 40 ml. pipettes, any milk adhering to within a depth of 1 in. of the mouth of the tube being charred in the Bunsen flame. The cotton-wool plugs in the mouths of the tubes were replaced with sterile tightly fitting rubber stoppers, through which passed short lengths of glass tubing ($\frac{1}{4}$ in. diam.) plugged with cotton-wool (Fig. 3). In addition, three stoppers were fitted with Class A N.P.L. thermometers reading to 0·2° F.

The filled tubes were placed in a water-bath maintained at 50 ± 1·0° F. (10·0° C.) for a minimum time of 10 min. Subsequently they were transferred in batches of three every 3 min. to a large electrically heated, thermostatically controlled water-bath (mercury-toluol), with forced water circulation, maintained between the limits of 146–147° F. (63·2–63·9° C.). The tubes were immersed to within half an inch of their mouths, buoyancy being overcome by means of small numbered weights (1–43) slipped over the glass tubing. The position of each tube in the bath was determined by drawing numbers out of a hat, the numbers drawn applying both to the number of the tube and its numbered position in the racks in the bath. Nos. 1, 21 and 43 were allotted to the tubes containing thermometers, which were placed in the bath at the commencement. The time of immersion of each tube was 35 min. The timing arrangements were made by means of an electric buzzer.

Immediately after pasteurization at 145·4 ± 0·4° F. (63·0 ± 0·2° C.) the tubes were transferred to a cold-water bath (approx. 50° F.) for 10 min., then to a $CaCl_2$ bath maintained at 25–30° F. (−3·9 to −1·1° C.). On completion of cooling each tube had its stopper removed, and after flaming the mouth, the contents were emptied into a sterile 2500 ml. wide-mouth Erlenmeyer flask. This flask was then stoppered with a sterile rubber stopper, shaken vigorously by hand for 3 min. and placed in the refrigerator until required.

(2) *Methods of examination*

Until required samples were kept in a refrigerator. Tests on raw milk samples were begun within 45 min. of taking. Experiments on the L.T. 'Holder' and H.T.S.T. pasteurized milk samples began 2½ hr. after the time of collection of the raw milk samples, because of the time taken to carry out laboratory pasteurization.

The following tests were used:

(i) *Titratable acidity*, using the method of Davis & Sadek (30) on all samples when fresh and on the two series of pasteurized milk samples after ageing them at 18° C. for 24 and 48 hr. In addition acidities at the end-points of K.Q. tests were recorded.

(ii) *Reduction tests.* M.B. and resazurin tests were set up in triplicate at 37 and 18° C. on the raw milk samples. Similar tests in triplicate were carried out at 18° C. on the L.T. 'Holder' and H.T.S.T. pasteurized milk samples when fresh, plus additional series (also in triplicate) at 37, 30 and 22° C. on the main bulk samples after they had been aged at 18° C. for 45½ hr. M.B. reduction tests were carried out by the method of Wilson (31), and the standard procedure (32, 33) was adopted for the resazurin tests. (The arbitrary time of ageing the pasteurized milk samples for 45½ hr. before setting up reduction tests conformed with the average time of storage of the commercially bottled pasteurized milk from the same dairy before being subjected to the statutory methylene-blue test (34).)

(iii) *Colony counts.* Plates were prepared in triplicate and incubated at temperatures of 37, 30 and 22° C. from the fresh raw and pasteurized milk samples and from the latter after ageing at 18° C. for 24 and 48 hr. Each series of nine plates poured was arranged in three groups, plate nos. 1, 5 and 9 being incubated at 37° C., nos. 2, 6 and 7 at 30° C., and nos. 3, 4 and 8 at 22° C. Plate counts were determined after incubation at 37, 30 and 22° C. for 3, 6 and 10 days respectively. Yeastrel milk agar (35) was used throughout, and the arithmetic means of the three counts at a particular dilution were recorded.

(iv) *Coliform tests.* MacConkey broth (35) tubes containing 1–0·000001 ml. amounts of

the raw milk samples were prepared in nines and arranged in three groups of three (as above) for incubation at 37, 30 and 22° C. respectively.

Tests were carried out in quintuplicate on 1 ml. amounts of the L.T. 'Holder' and H.T.S.T. pasteurized milk samples when fresh and after ageing at 18° C. for 24 and 48 hr. Three series of tests were made, series being incubated at 37, 30 and 22° C. for 3, 6 and 10 days respectively.

(v) *Keeping quality tests.* Alcohol and clot-on-boiling (C.O.B.) tests were carried out at half-hourly intervals on the 18° C. aged pasteurized milk samples after complete reduction in the M.B. and resazurin tests on samples aged for 45½ hr. at the same temperature.

Alcohol tests were made using the method described by Anderson & Wilson (36). C.O.B. tests were carried out by immersing 5 ml. of the sample contained in a $6 \times \frac{5}{8}$ in. test-tube in a bath of boiling water for 5 min., precipitation of the casein being regarded as a positive reaction.

(vi) *Phosphatase tests.* Neave's modification of the Kay & Graham test (37) was used on all L.T. 'Holder' and H.T.S.T. pasteurized milk samples when fresh to check the efficiency of heat treatment.

Experiments were carried out over a period of three years, and the milk examined was representative of tank milk of average commercial quality consigned to London. It was produced and bulked in Devonshire, the only conditions determining its acceptance and use for experimental purposes being:

(*a*) that the temperature on receipt at the pasteurizing depot did not exceed 40° F. (4·4° C.),

(*b*) that neither the laboratory L.T. 'Holder' nor H.T.S.T. pasteurized milk samples when submitted to the M.B. reduction test at 55° C. were decolorized in less than 6 hr.

Throughout the series of experiments every endeavour was made to maintain a uniform technique in sampling and testing; and only the results of complete experiments have been recorded.

EXPERIMENTAL RESULTS

A. *Pasteurization of fresh raw milk*

(1) *Colony counts*

The results of thirty-six experiments are summarized in Table 1.

The geometric mean counts at the three temperatures of incubation show that fewer bacteria survived the L.T. 'Holder' process. Plate counts of the freshly pasteurized milk samples were highest at 22° C. and lowest at 37° C. On the other hand, maximum geometric mean plate counts on the raw milk samples occurred at an incubation temperature of 30° C.

The rate of increase in counts as determined at intervals of 24 hr., was maintained steadily at all three temperatures of incubation of the plates in the case of the H.T.S.T. samples, whereas the L.T. 'Holder' samples increased slightly during the first 24 hr. interval, then rapidly in the 24–48 hr. period. The increase in numbers during the 24–48 hr. interval was much more rapid in the L.T. 'Holder' than in the H.T.S.T. samples.

By estimating the difference between the paired geometric mean counts in terms of one series of geometric means on a percentage basis, i.e. according to the formula

$$\left(\frac{A-B}{A}\right) \times 100,$$

where A = geometric mean plate count of the H.T.S.T. samples, and B = geometric mean plate count of the corresponding L.T. 'Holder' samples, the following results were obtained:

	Temperature of incubation of plates								
	37° C.			30° C.			22° C.		
Age of samples (hr. at 18° C.) ...	0	24	48	0	24	48	0	24	48
% increase L.T./H.T.	48·2	85·8	42·8	41·9	88·0	37·2	41·0	86·8	37·3

The similarity between these percentages at corresponding time intervals at all three temperatures of incubation suggests that a relationship exists between the numbers of bacteria which survive the two processes and the numbers which proliferate subsequently. The difference in the rates of multiplication in the 0–24 and 24–48 hr. ageing periods at 18° C. suggests different behaviour of the floras of the two pasteurized samples, which may in turn be related to some difference in the lethal effect of the two forms of heat treatment.

Table 1. *Colony counts of raw and low-temperature 'Holder' and high-temperature short-time pasteurized milk samples*

(Summarized results of thirty-six experiments)

	Age at testing (hr. at 18° C.)	Geometric means	Range of variation	
			Max.	Min.
Incubation temp. 37° C.:				
Raw	0	649,400	7,600,000	59,000
L.T. 'Holder'	0	10,090	96,700	1,160
	24	28,670	1,275,000	2,840
	48	720,700	87,300,000	1,520
H.T.S.T.	0	19,470	94,500	1,150
	24	202,600	5,700,000	4,700
	48	1,260,000	21,600,000	1,620
Incubation temp. 30° C.:				
Raw	0	2,985,000	25,900,000	242,000
L.T. 'Holder'	0	33,960	145,000	11,350
	24	64,710	1,610,000	14,100
	48	1,928,000	104,000,000	18,500
H.T.S.T.	0	58,430	243,000	3,200
	24	537,600	7,667,000	14,100
	48	3,070,000	26,900,000	23,750
Incubation temp. 22° C.:				
Raw	0	2,723,000	31,700,000	255,000
L.T. 'Holder'	0	35,480	183,700	11,900
	24	65,570	1,730,000	14,300
	48	1,849,000	96,300,000	21,100
H.T.S.T.	0	60,170	263,000	17,300
	24	497,800	10,500,000	31,000
	48	2,949,000	25,330,000	26,600

Table 2 sets out the correlation coefficients between the plate counts of the raw milk and the freshly pasteurized milk samples at the three temperatures of incubation. With the exception of the fresh L.T. 'Holder' samples, all r values are greater than those shown in Fisher's Table VA(38) for $P = 0{\cdot}05$, where $n = N - 2$, thus indicating a real degree of association between the paired variables.

The differences between the two series of r values are mainly:

(*a*) a lack of correlation between the raw and fresh L.T. 'Holder' pasteurized samples at all three plate incubation temperatures, suggesting a departure from normality, and

(*b*) the order of the r values between the raw and H.T.S.T. samples and those between the raw and aged L.T. 'Holder' samples, indicating a difference between the relative effects

Table 2

	Age of sample (hr.)	Plate incubation temperature 37° C.	30° C.	22° C.
A. Raw/L.T.	0	+0·01	+0·02	+0·16
	24	+0·49	+0·47	+0·49
	48	+0·38	+0·48	+0·40
B. Raw/H.T.	0	+0·59	+0·52	+0·59
	24	+0·59	+0·65	+0·75
	48	+0·55	+0·56	+0·53

Table 3

	Age of sample (hr.)	Plate incubation temperature 37° C.	30° C.	22° C.
A. L.T.	0/24	+0·27	+0·27	+0·36
	24/48	+0·67	+0·61	+0·54
B. H.T.	0/24	+0·70	+0·73	+0·75
	24/48	+0·80	+0·71	+0·69

Table 4

	Age of sample (hr.)	Plate incubation temperature 37° C.	30° C.	22° C.
L.T./H.T.	0	+0·49	+0·32	+0·36
	24	+0·51	+0·52	+0·55
	48	+0·71	+0·72	+0·73

Table 5

	Age of sample (hr.)	Plate incubation temperature 37° C./30° C.	37° C./22° C.	30° C./22° C.
A. L.T.	0	+0·75	+0·66	+0·89
	24	+0·89	+0·85	+0·24
	48	+0·92	+0·94	+0·95
B. H.T.	0	+0·78	+0·75	+1·00
	24	+0·92	+0·91	+0·99
	48	+0·93	+0·92	+0·97
C. Raw	0	+0·88	+0·95	+0·89

of the two forms of heat treatment. A possible explanation of these differences is as follows:

The effect of H.T.S.T. pasteurization on the growth and development of the surviving microflora is negligible, in that numbers increase systematically during the ageing period. Hence the numbers of bacteria present in the freshly pasteurized milk samples can be expected to show a good relationship to those present after ageing for 24 hr. Similarly, the correlation coefficients between the numbers at the 24 and 48 hr. intervals should be equally good. Data in Table 3B support this supposition.

The L.T. 'Holder' process, however, appears to depress the growth of survivors, so much so that only after ageing for 24 hr. do they multiply normally. If this is the case, it may well be that the plate counts of freshly pasteurized L.T. 'Holder' samples are inaccurate, owing to the inability of some bacteria to grow in an artificial substrate and manifest themselves as countable colonies. The fact that the 24 and 48 hr. plate counts of the L.T. 'Holder' samples are related to the corresponding raw milk counts tends to confirm the assumption that the lag phase of growth of certain bacteria in the fresh samples occupies a longer time than the period of incubation of the plates. The contrast between 0, 24 and 48 hr. correlation coefficients in Tables 2A and 2B, and comparison between the results

in Tables 3A and 3B, also support these hypothetical differences between the effects of the two methods of pasteurization.

Further consideration of the results in Table 3 shows that there is a closer relationship between the numbers of viable organisms in the fresh and aged H.T.S.T. samples than in corresponding L.T. 'Holder' samples. It is also noteworthy that the r values at different time intervals, and at different plate incubation temperatures, show only limited variation in each series of samples, thus indicating proportionate increases in bacterial numbers in the respective samples as they deteriorate.

A means of comparing the processes of deterioration in the two series of pasteurized samples in terms of bacterial numbers is afforded by Table 4. These results show a progressive increase in correlation coefficients from 0 to 48 hr. at all three plate incubation temperatures, which appears to be evidence that there is a more and more marked similarity between the processes of deterioration in the two series of samples as ageing proceeds.

The uniformly high correlation coefficients shown in Table 5 point to a close relationship between the plate counts on each series of pasteurized milk samples at each time interval. It is significant that there is little difference between the order of the raw, L.T. 'Holder' and H.T.S.T. r results.

A further point of interest is that the correlation coefficients in Table 5 exceed those in Table 4. This suggests that there is a much closer relationship between the three plate counts at each of the three 24 hr. intervals, than between successive plate counts at each of the three plate incubation temperatures. Thus although successive plate counts increase and follow a general pattern, the ratios of the 30 and 22° C. to the 37° C. plate counts at each time interval tend to be similar, viz.

		Plate count ratios							
Description ...		Raw		Pasteurized (L.T. 'Holder', H.T.S.T.)					
Age of samples (hr. at 18° C.) ...		0		0		24		48	
Incubation temp. (° C.) ...		30	22	30	22	30	22	30	22
Arithmetic means of thirty-six results		5·26	6·09	4·67	4·68	3·05	3·20	3·62	3·76
				4·06	**4·01**	**3·70**	**4·02**	**4·28**	**4·54**

Heavy type = H.T.S.T.

In certain experiments plate counts decreased when samples were aged at 18° C. Such decreases were noted in eleven L.T. 'Holder' and in four H.T.S.T. samples, and suggest an alteration in flora which can only be explained by the death of some bacteria, as a consequence of either the heating process or a change in environmental conditions. The fact that it seemed more liable to occur in the L.T. 'Holder' than the H.T.S.T. samples is perhaps noteworthy.

(2) *Percentage destruction of bacteria*

The arithmetic mean percentage destruction of bacteria at each of the three incubation temperatures of the plates for the thirty-six parallel samples was as follows:

Temperature of incubation of plates ...		37° C.	30° C.	22° C.
Age of samples (hr.) ...		0	0	0
Arithmetic means of thirty-six results	L.T.	96·48	97·34	97·83
	H.T.S.T.	**95·11**	**96·67**	**97·09**

These figures show that the greatest numbers of bacteria are viable at 22° C. and successively at 30 and 37° C. The divergence between survival rates is greatest at 37° C. and least at 30° C., the difference between the 30 and 22° C. rates being negligible.

In individual experiments there was good agreement between the corresponding L.T. 'Holder' and H.T.S.T. destruction percentages, but in all cases the L.T. 'Holder' figures were slightly higher. This indicates that the two processes are slightly dissimilar, the thermal destruction rate being higher for the L.T. 'Holder' method.

(3) *Coliform tests*

The numbers of samples showing acid and gas reactions in one, two and in occasional cases three of each series of five MacConkey broth tubes at any of the three incubation temperatures used, irrespective of whether these reactions were obtained from the fresh or 18° C. aged samples, was limited to eight L.T. 'Holder' and thirteen H.T.S.T. samples out of the thirty-six examined. Attempts to reproduce these apparent positive reactions by inoculating sterile MacConkey broth with four loopfuls of inoculum, as soon as acid and gas was clearly evident, followed by incubation at the same temperatures, yielded uniformly negative results. Direct plating from the same tubes on Levine's eosine methylene-blue agar (39), and incubation of additional tubes at 44° C., were equally unsuccessful. It was therefore concluded that coliform organisms were absent from 1 ml. quantities of the fresh and aged pasteurized milk samples, and that the presence of acid and gas in single, duplicate and triplicate MacConkey broth tubes was a false reaction. It is of some interest to note that false positive reactions occurred to the greatest extent in aged samples and in tubes incubated at 37° C.

In the raw milk samples there was a tendency for presumptive positives to occur at lower dilutions in the tubes incubated at 22° C., compared with those incubated at 30 and 37° C.

(4) *Reduction tests, alcohol precipitation tests and clot-on-boiling tests as a means of determining keeping quality*

On the whole the methylene-blue and resazurin tests carried out at 18° C. on the freshly pasteurized milk samples yielded unsatisfactory results. Complete decolorization of either or both the triplicate methylene-blue or resazurin tubes showed considerable variability, so much so that definite end-point readings were often unobtainable. This frequent lack of uniformity between triplicate resazurin tubes (especially between disk readings of $4\frac{1}{2}$ and 0) and the difference in the poising effect (which was most pronounced at disk $\frac{1}{2}$ and was also evident in some series of methylene-blue tubes on the point of reduction) suggest that the intensity of the reducing system in 1 ml. quantities of freshly pasteurized milk incubated at 18° C. is liable to variation. The reasons for this are discussed later.

The results of reduction tests carried out at 37, 30 and 22° C. on the original samples of the bulk pasteurized milks, which had been aged for $45\frac{1}{2}$ hr. at 18° C., may be summarized as follows (the results also include the alcohol and clot-on-boiling tests to end-points at 18° C.):

Description ...	L.T. 'Holder'			H.T.S.T.		
	Max.	Min.	Mean	Max.	Min.	Mean
M.B.R.T. (hr.) at:						
37° C.	4·0	0·5	1·7	6·0	0·5	2·2
30° C.	5·5	0·5	2·5	9·0	1·0	3·5
22° C.	9·5	1·0	4·7	10·5	1·5	5·9
68% Alcohol + (hr.)	76·0	46·5	60·0	79·5	48·0	65·5
C.O.B. + (hr.)	94·0	48·5	68·0	98·0	53·0	78·5
Difference between means of 68% alcohol and C.O.B. tests (hr.)	18·0	2·0	8·0	18·5	5·0	13·0

Unlike reduction tests on the freshly pasteurized milk samples, these triplicate tests on aged samples showed only very occasional diversity. In each experiment at least two of each series of three tubes were completely reduced at the same time interval. Moreover, the rates of reduction as denoted by the hourly resazurin disk readings were comparable. The poising effect at disk ½ was noted on occasions but was of less duration, and only occurred infrequently at the lower incubation temperatures. This agrees with previous findings of Rowlands & Provan (40) that an ageing period is essential when dye reduction tests are applied to pasteurized milk. Corresponding complete reduction times of resazurin and methylene blue at each of the three temperatures of incubation showed a marked similarity. In general, the tubes containing resazurin were decolorized before those containing methylene blue.

Table 6

Tests	Correlation coefficients (r) L.T.	H.T.
1. Alcohol/C.O.B.	+0·84	+0·86
2. M.B. 37° C./C.O.B.	+0·49	+0·42
M.B. 30° C./C.O.B.	+0·51	+0·59
M.B. 22° C./C.O.B.	+0·63	+0·58
3. M.B. 37° C./Alcohol	+0·54	+0·48
M.B. 30° C./Alcohol	+0·57	+0·63
M.B. 22° C./Alcohol	+0·67	+0·60
4. M.B. 37° C./M.B. 30° C.	+0·77	+0·83
M.B. 37° C./M.B. 22° C.	+0·73	+0·65
M.B. 30° C./M.B. 22° C.	+0·74	+0·81
5. Plate count 0 hr. 37° C./C.O.B.	−0·24	+0·44
Plate count 24 hr. 37° C./C.O.B.	+0·14	+0·56
Plate count 48 hr. 37° C./C.O.B.	+0·14	+0·45
6. Plate count 0 hr. 37° C./Alcohol	−0·16	+0·44
Plate count 24 hr. 37° C./Alcohol	−0·04	+0·43
Plate count 48 hr. 37° C./Alcohol	0·00	+0·02
7. Plate count 0 hr. 37° C./M.B. 37° C.	−0·61	−0·16
Plate count 24 hr. 37° C./M.B. 37° C.	−0·31	+0·02
Plate count 48 hr. 37° C./M.B. 37° C.	−0·48	+0·10
8. Plate count 0 hr. 30° C./C.O.B.	−0·14	+0·19
Plate count 24 hr. 30° C./C.O.B.	+0·20	+0·33
Plate count 48 hr. 30° C./C.O.B.	+0·11	+0·30
9. Plate count 0 hr. 30° C./Alcohol	+0·04	−0·06
Plate count 24 hr. 30° C./Alcohol	−0·03	+0·14
Plate count 48 hr. 30° C./Alcohol	−0·04	+0·04
10. Plate count 0 hr. 30° C./M.B. 30° C.	−0·08	+0·15
Plate count 24 hr. 30° C./M.B. 30° C.	−0·24	+0·07
Plate count 48 hr. 30° C./M.B. 30° C.	−0·34	−0·02
11. Plate count 0 hr. 22° C./C.O.B.	−0·07	+0·21
Plate count 24 hr. 22° C./C.O.B.	+0·16	+0·31
Plate count 48 hr. 22° C./C.O.B.	+0·12	+0·31
12. Plate count 0 hr. 22° C./Alcohol	+0·01	0·00
Plate count 24 hr. 22° C./Alcohol	−0·02	+0·17
Plate count 48 hr. 22° C./Alcohol	−0·04	+0·04
13. Plate count 0 hr. 22° C./M.B. 22° C.	−0·20	+0·32
Plate count 24 hr. 22° C./M.B. 22° C.	−0·26	+0·15
Plate count 48 hr. 22° C./M.B. 22° C.	−0·19	+0·09
14. C.O.B. tests—L.T./H.T.	+0·42	
15. Alcohol tests—L.T./H.T.	+0·55	
16. M.B. tests at 37° C.—L.T./H.T.	+0·23	
17. M.B. tests at 30° C.—L.T./H.T.	+0·27	
18. M.B. tests at 22° C.—L.T./H.T.	+0·42	

Table 6 indicates the degree of association between the various tests applied to the

pasteurized milk samples. Owing to the similarity between the two reduction tests, only the methylene-blue results have been used. The results show that relationships exist between the methylene-blue, alcohol and C.O.B. tests. The *r* values obtained for the alcohol/C.O.B. tests were found to be appreciably higher than either the M.B./alcohol or M.B./C.O.B. comparisons and may indicate the relative value of the three tests as a means of assessing the keeping quality of pasteurized milk.

It is interesting to note that off-flavours or taints in the pasteurized milk samples were detectable at approximately the same times as samples were positive to C.O.B. tests. The onset of these changes, which in the early stages were variously described as 'flat', 'tasteless', 'chalky', 'watery', etc., was difficult to determine and considered unreliable as a means of estimating K.Q. for comparative purposes.

The correlation coefficients between the methylene-blue tests at 22 and at 30° C., and the alcohol and C.O.B. tests for both pasteurized milk samples were better than the correlation coefficients between reduction tests at 37° C. and the other end-point tests.

The relationship between the methylene-blue tests at the three temperatures of incubation was good. The similarity between the *r* values on the L.T. 'Holder' pasteurized milks appears to indicate that the active reducing flora is common to all three temperatures of incubation, the only difference being in the speed of the reactions. On the other hand, the correlation coefficients for the H.T.S.T. pasteurized milk samples seem to indicate slight differences in this respect, the reducing flora being more similar and active at temperatures of 30 and 22° C.

The plate counts at 37, 30 and 22° C. on the fresh and aged pasteurized milk samples were on the whole poorly correlated with the methylene-blue, alcohol and C.O.B. tests. This lack of association between the plate counts, and the methylene-blue reduction, alcohol and C.O.B. tests, is evidence that specific types or groups of bacteria, and not total numbers, are responsible for the processes of deterioration which ultimately result in souring. From the titratable acidities of samples taken at the keeping quality test end-points (summarized below), it was obvious that clotting in both series of pasteurized milk samples was due invariably to the activity of proteolytic organisms.

	Titratable acidity (% L.A.)								
	Raw			L.T. 'Holder'			H.T.S.T.		
	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
0 hr.	0·170	0·145	0·154	0·160	0·140	0·147	0·160	0·140	0·148
24 hr. (aged 18° C.)	—	—	—	0·165	0·140	0·151	0·165	0·145	0·154
48 hr. (aged 18° C.)	—	—	—	0·180	0·150	0·163	0·195	0·145	0·162
37° C. M.B.R.T. (45½ hr. test on 18° C. aged samples)	—	—	—	0·185	0·150	0·169	0·195	0·150	0·167
68% alcohol +	—	—	—	0·190	0·160	0·181	0·195	0·160	0·180
C.O.B. +	—	—	—	0·210	0·175	0·191	0·215	0·165	0·195

B. *Pasteurization of aged raw milk*

The results so far recorded were sufficient to indicate certain trends in the behaviour of corresponding L.T. 'Holder' and H.T.S.T. pasteurized milk samples when they were aged at 18° C. Further work of a more detailed nature, however, appeared necessary to explain the slight differences between the effects of the two forms of heat treatment.

In order to accentuate any differences it was decided to obtain and examine a first

series of raw and pasteurized milk samples in the manner described previously, then to age the bulked raw milk at 40–50° F. (4·4–10·0° C.) for 24 hr. and subsequently examine this second series of parallel raw and L.T. 'Holder' and H.T.S.T. pasteurized milk samples.

Deterioration of the samples was followed by making statistical picks from the incubated plates according to the method of Harrison(41), followed by inoculating the organisms into litmus milk and noting the reactions on incubation. Approximately thirty colonies per plate were transferred into litmus milk, cultures being incubated at the same temperature as the original plates from which they were obtained. The changes during incubation were noted every 2 days up to 30 days.

Attempts to classify the organisms on the basis of their morphology proved too cumbersome, but certain bacteria which appeared to be of special significance were examined in detail, and where possible identified.

(1) *Plate counts*

The results of seven experiments have been arranged in two groups (Table 7), according to the 37° C. plate counts of the fresh raw-milk samples. Group 1 comprises the arithmetic means for three series of samples, the remaining four being grouped in Group 2.

The arithmetic mean plate counts of the raw, L.T. 'Holder', and H.T.S.T. pasteurized milk samples confirm the previous findings and in addition suggest that:

(*a*) Bacterial multiplication during ageing of raw milk for 24 hr. at 40–50° F. (4·4–10·0° C.) is mainly limited to the growth of heat-destructible organisms, since the plate counts of the freshly pasteurized milks were very similar for both fresh and aged raw milk. This also accounts for the increase in the percentage destruction of bacteria on pasteurization after ageing the raw milk.

(*b*) There is a relationship between the plate counts of the fresh raw milk samples and the rate of increase in the plate counts of the pasteurized milk samples at intervals of 24 hr. during storage at 18° C. Results appeared to indicate that the higher the plate counts of the fresh raw milk the greater the rate of increase in count of the L.T. 'Holder' samples during the 24–48 hr. storage period and more rapid the progressive increase in count of the H.T.S.T. samples throughout the 48 hr. period.

(2) *Coliform tests*

Coliform organisms were absent from 1 ml. amounts in both the L.T. 'Holder' and H.T.S.T. pasteurized milk samples. The presence of acid and gas in certain MacConkey broth tubes in three pasteurized milk samples (one L.T. 'Holder' and two H.T.S.T.) was noted, but attempts to reproduce the same reactions by subculturing failed. Microscopic examination of several of these false positives revealed the presence of long-chained streptococci, which grew rapidly and produced acid and gas in MacConkey broth incubated at 44° C.

All the raw milk samples gave positive coliform reactions. Incubation temperatures of 30 and 22° C. appeared to favour the maximum development of coliform organisms in the raw milk samples, as they were usually present in higher dilutions at these temperatures than at 37° C. It is also of some interest to note that growth of coliforms occurred in bulk raw milk during the 24 hr. ageing within the range of 40–50° F. (4·4–10·0° C.).

Table 7. *Arithmetic mean plate counts of raw and low-temperature 'Holder' and high-temperature short-time pasteurized milk samples*

Arith. mean colony count at	Age of samples (hr.)	Ageing temp. (° C.)	Raw			L.T. 'Holder'			H.T.S.T.		
			Group 1	Group 2	Combined groups	Group 1	Group 2	Combined groups	Group 1	Group 2	Combined groups
37° C.	A. 0	—	237,000	143,600	183,000	—	—	—	—	—	—
	0	—	—	—	—	6,700	9,500	8,300	7,100	10,000	8,800
	24	18	—	—	—	7,200	9,600	8,600	11,100	9,200	10,000
	48	18	—	—	—	449,500	10,300	197,600	243,100	15,400	110,800
	B. 24	4·4–10·0	311,300	189,800	214,900	—	—	—	—	—	—
	0	—	—	—	—	5,500	8,600	7,300	6,400	16,000	11,900
	24	18	—	—	—	5,100	11,500	8,800	175,000	31,200	92,800
	48	18	—	—	—	108,300	38,700	68,500	272,300	159,000	207,500
30° C.	A. 0	—	1,877,000	470,000	1,073,300	—	—	—	—	—	—
	0	—	—	—	—	42,600	34,200	37,800	37,500	34,800	35,900
	24	18	—	—	—	33,500	29,400	31,200	95,100	29,000	57,400
	48	18	—	—	—	2,398,300	29,600	1,044,700	1,669,000	29,700	732,000
	B. 24	4·4–10·0	5,543,000	1,683,800	3,337,000	—	—	—	—	—	—
	0	—	—	—	—	25,300	24,500	24,800	29,200	28,100	28,600
	24	18	—	—	—	26,800	27,900	26,900	76,400	39,700	55,500
	48	18	—	—	—	542,800	31,500	250,000	3,045,700	128,900	1,378,900
22° C.	A. 0	—	985,000	597,300	763,400	—	—	—	—	—	—
	0	—	—	—	—	39,300	29,400	33,600	29,900	27,900	28,800
	24	18	—	—	—	32,600	25,900	28,700	70,600	32,300	48,700
	48	18	—	—	—	2,326,700	25,100	1,011,500	1,799,000	30,700	788,600
	B. 0	4·4–10·0	5,530,000	1,890,000	3,450,000	—	—	—	—	—	—
	0	—	—	—	—	25,100	25,100	25,100	30,700	30,400	30,500
	24	18	—	—	—	30,700	27,200	28,700	164,100	41,500	94,000
	48	18	—	—	—	544,800	29,900	250,600	4,655,000	125,600	2,066,800

A = tests carried out on bulked raw milk and corresponding pasteurized milk samples.

B = tests carried out on bulked raw milk aged for 24 hr. at 40–50° F. (4·4–10·0° C.) and corresponding pasteurized milk samples.

(3) *Reduction tests, alcohol precipitation tests, and clot-on-boiling tests as a means of determining keeping quality*

A series of keeping quality tests on the fresh and aged raw and corresponding pasteurized milk samples similar to those previously described was carried out, and the results are summarized in Table 8. Corresponding with this table, the percentage reductions in keeping quality based on the paired results of identical tests are summarized in Table 9.

Table 8. *Arithmetic mean results of keeping quality tests on raw and low-temperature 'Holder' and high-temperature short-time pasteurized milk samples*

	Tests	Raw			L.T. 'Holder'			H.T.S.T.		
		Group 1	Group 2	Combined groups	Group 1	Group 2	Combined groups	Group 1	Group 2	Combined groups
A.	M.B.R.T. (hr.) at 37° C.	2·5	4·6	3·7	2·0	3·0	2·6	2·7	4·0	3·4
	M.B.R.T. (hr.) at 30° C.	3·0	4·9	4·1	2·8	3·4	3·1	3·8	5·1	4·6
	M.B.R.T. (hr.) at 22° C.	—	7·8	—	—	—	—	—	—	—
	M.B.R.T. (hr.) at 18° C.	6·3	11·6	9·4	7·3	8·9	8·2	8·5	11·6	10·3
	68% alcohol + (hr.)	12·5	16·9	15·0	56·8	56·0	56·4	63·7	68·0	66·1
	C.O.B. + (hr.)	17·5	23·1	20·7	59·3	60·6	60·1	67·8	74·6	71·7
B.	M.B.R.T. (hr.) at 37° C.	1·3	3·4	2·5	1·2	1·6	1·4	1·5	2·4	2·0
	M.B.R.T. (hr.) at 30° C.	1·3	3·5	2·6	1·8	2·1	2·0	2·5	3·3	2·9
	M.B.R.T. (hr.) at 22° C.	—	4·6	—	—	—	—	—	—	—
	M.B.R.T. (hr.) at 18° C.	2·7	7·0	5·1	5·3	5·8	5·6	7·2	7·3	7·2
	68% alcohol + (hr.)	8·3	13·3	11·1	51·7	50·5	51·0	59·2	58·1	58·6
	C.O.B. + (hr.)	13·5	21·1	17·9	54·0	55·3	54·7	63·3	65·3	64·4

A = tests carried out on bulked raw milk and corresponding pasteurized milk samples.

B = tests carried out on bulked raw milk aged for 24 hr. at 40–50° F. (4·4–10·0° C.) and corresponding pasteurized milk samples.

M.B.R.T. on all pasteurized milk samples were carried out after incubation of bulked samples for 45½ hr. at 18° C.

Table 9. *Percentage reduction in test results corresponding to Table* 8

	M.B.R.T. at					
	37° C.	30° C.	22° C.	18° C.	Alcohol +	C.O.B. +
			Raw milk			
Group 1	48·0	56·7	—	57·1	33·6	22·9
Group 2	26·0	28·6	41·0	39·7	21·3	8·7
Mean	32·4	36·6	—	45·7	26·0	13·5
			L.T. samples			
Group 1	40·0	35·7	—	27·4	9·0	8·9
Group 2	46·7	38·2	—	34·8	9·8	8·7
Mean	46·2	35·5	—	31·7	9·6	9·0
			H.T. samples			
Group 1	44·4	34·2	—	15·3	7·1	6·6
Group 2	40·0	35·3	—	37·1	14·6	12·5
Mean	41·2	37·0	—	30·1	11·3	10·2

The results show that there is a decrease in keeping quality when raw milk is aged for 24 hr. at 40–50° F. (4·4–10° C.) and that L.T. 'Holder' and H.T.S.T. samples corresponding to the same fresh and aged raw milk are affected similarly when samples are aged at 18° C. It would appear that there is little if any correlation between the relative rates of deterioration of the raw and pasteurized samples. On the other hand, there was a similarity between individual pairs of L.T. 'Holder' and H.T.S.T. pasteurized milk results, which suggests that the processes of deterioration are comparable although the rates are different.

The corresponding pairs of pasteurized milk samples showed significant differences in keeping quality, the H.T.S.T. samples produced from the fresh and aged raw milk exceeding that of the L.T. 'Holder' samples by 19·3 and 17·7% respectively.

From these experimental results evidence has been accumulated which tends to indicate that the numbers and activity of heat-resistant organisms which constitute the flora of either the L.T. 'Holder' or H.T.S.T. pasteurized milk samples are not related to the viable numbers or biological activity of the organisms in the corresponding raw milk samples, as gauged by plate counts or reduction tests. Hence, the bacteriological results obtained on examination of bulked raw milk are of little guidance in estimating the probable keeping quality of the same milk, when it is carefully pasteurized by either the L.T. 'Holder' or H.T.S.T. methods.

A few additional experiments on pasteurized milk samples were made using ethyl alcohol at 60 and 50% strength (v/v) to estimate keeping quality. 68% alcohol and C.O.B. tests were also carried out, the latter being taken as a standard. In fifty experiments the times required to reach the end-points of the alcohol and C.O.B. tests approached one another as the strength of the alcohol solution decreased, viz.

	Alcohol tests (hr.)			C.O.B. tests
	68%	60%	50%	(hr.)
Arith. means (50 tests)	70·9	71·8	73·0	74·4

In this respect raw and pasteurized milk appear to be similar.

As in the previous series of samples, it was noted that end-points usually occurred at low titratable acidities, suggesting that the instability of the pasteurized milk samples to alcohol or heat was mainly, if not entirely, due to the effects of proteolysis rather than to acid formation.

(4) *Bacterial flora of samples*

The final reactions of litmus milk cultures of colonies isolated from incubated plates were classified under the following headings: acid, acid with clot, alkaline, peptonizing, inert and no growth. Summarized results are given in Table 10. The following are brief notes on the changes in microflora in the raw milk aged for 24 hr. at 40–50° F. (4·4–10·0° C.), and in the corresponding L.T. 'Holder' and H.T.S.T. pasteurized milk samples aged at 18° C., as indicated by the reactions observed.

(i) *Raw milk.* The acid-forming groups predominated in both fresh and aged samples at all three plate-incubation temperatures. The highest proportion of acid-forming organisms grew on the plates incubated at 37° C. The alkali-producing and peptonizing groups occurred at all three incubation temperatures but showed a tendency to form a greater percentage of the total flora at the two lower temperatures. This feature was more noticeable in the case of the peptonizers.

The inert group showed the most marked development at 37° C., and apparently constituted a slightly higher proportion of the flora in the aged samples. Of the acid-forming species, those producing a clot occurred to the greatest extent on the 37° C. plates, whereas the non-clotting types were present in the greatest numbers on the 22° C. plates.

The ageing of raw milk at 40–50° F. (4·4–10·0° C.) resulted in a stimulation of the peptonizing group, the growth of these species being most noticeable on the 30 and 22° C. plates. At these temperatures the proportion of acid-formers decreased, the acid-

Table 10. *Analysis of litmus milk reactions—combined results of fresh and aged samples*

Litmus milk reactions	Temperature of incubation of litmus milk cultures: 37° C. Acid	Acid-clot	Alkaline	Peptonizing	Inert	No growth	30° C. Acid	Acid-clot	Alkaline	Peptonizing	Inert	No growth	22° C. Acid	Acid-clot	Alkaline	Peptonizing	Inert	No growth
Raw milk (age of milk from which cultures obtained):																		
0 hr.	8·7	70·3	6·7	6·0	8·3	—	26·5	46·4	2·0	20·3	4·0	0·7	30·8	35·0	10·0	21·7	2·5	—
L.T. 'Holder' (age of milk from which cultures obtained):																		
0 hr.	14·7	74·7	—	3·3	7·3	—	24·0	70·0	2·7	2·7	0·7	—	54·1	40·4	2·7	1·6	1·1	—
24 hr.	21·2	63·0	3·4	2·1	8·9	1·4	21·2	71·5	2·6	3·3	1·3	—	49·1	40·7	7·8	2·4	—	—
48 hr.	7·2	21·6	8·4	55·7	6·6	0·6	19·0	29·6	12·7	34·9	3·7	—	21·5	14·2	5·6	58·2	0·6	—
H.T.S.T. (age of milk from which cultures obtained):																		
0 hr.	22·3	70·3	—	3·4	4·0	—	43·2	51·1	1·4	2·2	2·2	—	49·3	46·1	2·0	0·7	2·0	—
24 hr.	23·3	45·3	20·7	4·0	6·7	—	27·0	59·9	9·9	2·6	0·7	—	46·1	34·2	14·5	3·9	1·3	—
48 hr.	13·2	28·1	22·7	31·8	4·2	—	35·0	27·0	8·8	27·0	2·2	—	29·2	14·9	13·0	42·2	0·6	—

formers producing a clot showing the greater percentage reduction. The proportion of peptonizers to other groups was higher in the better quality than in the poorer quality milks. In general ageing increased the percentage of peptonizers and decreased the proportion of acid-formers. This effect was more pronounced in the better quality samples, suggesting that acid-forming organisms exercise some restraining influence on the growth of peptonizers when raw milk samples are aged at low temperatures.

(ii) *Pasteurized milk.* The process of deterioration in the L.T. 'Holder' and H.T.S.T. pasteurized milk samples seemed to be similar to the changes which occurred in the raw milk samples, i.e. there was an increase in the proportion of peptonizers, accompanied by a corresponding decrease in the percentage of acid-forming organisms.

The most noticeable difference between parallel series of samples was the alteration in group proportions as the samples deteriorated. The H.T.S.T. samples showed a comparatively slow reduction in acid-formers, which corresponded with a simultaneous slow increase in the proportion of peptonizers, during the two successive 24 hr. periods. The greatest increases and corresponding decreases occurred during the 24–48 hr. period, so much so that the peptonizers were becoming predominant, and would be expected to cause curdling within a limited space of time. This time would be determined in all probability, not only by total numbers, but by the numbers of certain species, and consequently their activity.

By comparison the L.T. 'Holder' samples showed only slight changes during the first 24 hr. of the ageing period, but marked alterations in the subsequent 24 hr. During the latter interval there was a considerable reduction in acid-formers, and an equally rapid increase in peptonizers, to the extent that the percentage of the latter group, relative to other groups, was much higher in the L.T. 'Holder' than in the corresponding H.T.S.T. pasteurized milk samples.

Comparison of the group proportions in the pasteurized milk samples, corresponding with the fresh and aged raw milk, seemed to indicate that the ratio of the proportion of

acid-formers to peptonizers increased at a much quicker rate in the pasteurized milk produced from the aged raw milk samples than in that from the fresh samples. This relationship between the raw and pasteurized milk samples suggests a change in the thermoduric flora of the fresh and aged raw milk samples, induced by the temperature of storage.

Some of the more commonly occurring organisms on the agar plates were isolated, purified, and identified, using Bergey's nomenclature (42). Tabulated results appear in Table 11.

DISCUSSION

(1) *Plate counts*

Explanations of the processes which lead to the deterioration of pasteurized milk in terms of colony counts at 37, 30 and 22° C. are likely to be very misleading. The experimental results have shown that the numbers of viable organisms determined by colony counts on yeastrel milk agar at these temperatures of incubation are not related to the lengths of time the L.T. 'Holder' or H.T.S.T. pasteurized milk samples remained sweet. Accordingly, plate counts can only be regarded as arbitrary and a means of comparing the total numbers of organisms at a particular time, or, as in this case, at successive intervals of time.

The results have also shown that ageing of raw milk at 40–50° F. (4·4–10·0° C.) caused appreciable increases in the colony counts at 37, 30 and 22° C., but that such increases were not accompanied by similar increases in the thermoduric colony counts of the corresponding L.T. 'Holder' or H.T.S.T. pasteurized milk samples; in fact, some decreases were noted. As both the L.T. 'Holder' and H.T.S.T. pasteurized milk samples produced from the aged raw milk showed a decrease in keeping quality, it must be concluded that changes occurred in the thermoduric microflora of the bulk raw milk during the 24 hr. storage period.

The experimental results have shown that milk pasteurized by the H.T.S.T. method has a higher viable thermoduric content than similar milk which has been subjected to the L.T. 'Holder' process. These findings are in accord with the published work of most other workers. The difference when colony counts are low is debatable, and a statistical examination of large numbers of parallel samples would be necessary to prove its existence. In the absence of such an analysis, the conclusion reached by Hiscox (43), that the two processes are equally bactericidal, must be open to doubt.

An interesting feature of the results recorded in this work was the lack of correlation between the colony counts of the raw and fresh L.T. 'Holder' pasteurized milk samples. As already indicated, the significant relationships between subsequent plate counts of the 18° C. aged samples and the raw milk samples, suggests that some of the micro-organisms present were unable to grow and appear as colonies on the plates. Hence it might be considered that the colony counts at 37, 30 and 22° C. of the fresh L.T. 'Holder' pasteurized milk samples were erroneous and not comparable with those of the fresh H.T.S.T. samples, since the latter gave r values which were significant.

It would appear that L.T. 'Holder' and H.T.S.T. pasteurized milk samples exhibit marked differences in behaviour, not only when they are fresh, but as they deteriorate when aged at 18° C. This difference seems to be related to a fundamental difference in the effects of the time/temperature conditions of the two processes. In the L.T. 'Holder'

Table 11. *Species of bacteria isolated from yeastrel milk-agar plates*

A. Raw milk samples

	Plate-incubation temperatures		
	37° C.	30° C.	22° C.
Streptococcus lactis types	+ + + +	+ + + +	+ + + +
Coliform organisms	+ +	+ +	+ +
Other species:			
Streptococcus pyogenes	+ +	+	+
Staphylococcus aureus	+	+	+
Lactobacillus casei	+	–	–
Micrococcus epidermis	–	+	+
Alcaligenes faecalis	–	(+)	(+)
Corynebacterium bovis	(+)	–	–
C. segmentosum	(+)	–	–
Pseudomonas sp. (incl. *Ps. ureae* (?))	–	–	(+)
Achromobacter butyrii	–	–	(+)
Aerobic spore-forming rods (*B. subtilis, B. mycoides*)	–	(+)	(+)

B. L.T. 'Holder' pasteurized milk samples

	Plate-incubation temperatures								
	37° C.			30° C.			22° C.		
Age of samples (hr. at 18° C.) ...	0	24	48	0	24	48	0	24	48
Streptococci (chiefly *S. faecalis, S. bovis*)	+ + + +	+ +	+ +	+ +	+	+	+ +	+	+
Lactobacilli (chiefly *L. plantarum, L. lactea, L. lactis*, less frequently *L. brevis*)	+	+	(+)	+	+	(+)	+	(+)	–
Microbacteria (chiefly *Mbm. lacticum, Mbm. flavum, Mbm. liquefaciens*)	–	–	–	+ + + +	+ + +	+ +	+ + + +	+ + +	+ +
Micrococci (chiefly *M. varians, M. aurantiacus, M. subcitreus, M. epidermis, M. luteus, M. flavus*)	+	+ +	+ +	+	+ +	+	+ +	+ +	+
Aerobic spore-forming rods (chiefly *B. subtilis, B. mycoides*, occasionally *B. brevis*)	–	+	+ + + +	–	+ +	+ + + +	–	+ +	+ + +
Miscellaneous:									
Sarcinae (*S. lactea*)	–	–	–	(+)	(+)	(+)	–	–	–
Achromobacter (*A. ubiquitum*)	–	–	–	–	–	(+)	–	–	–
Alcaligenes (*A. metalcaligenes*)	–	–	–	–	–	–	–	(+)	(+)

Table 11 (*continued*)

C. H.T.S.T. pasteurized milk samples

	Plate-incubation temperatures								
	37° C.			30° C.			22° C.		
Age of samples (hr. at 18° C.) ...	0	24	48	0	24	48	0	24	48
Streptococci (chiefly *S. faecalis, S. bovis*)	+ + + +	+ + +	+ +	+ +	+ +	+	+ +	+ +	+
Lactobacilli (chiefly *L. plantarum, L. casei, L. lactis*)	+	+	(+)	+	+	(+)	(+)	–	–
Microbacteria (chiefly *Mbm. lacticum, Mbm. liquefaciens, Mbm. flavum*)	–	–	–	+ + + +	+ + +	+ +	+ + + +	+ + +	+ +
Micrococci (chiefly *M. varians, M. freudenreichii, M. perflavus, M. aurantiacus, M. caseolyticus, M. viscosus, M. epidermis, M. luteus, M. subcitreus*, occasionally *M. ureae, M. candicans*)	+ +	+ +	+	+ +	+ +	+	+ +	+ +	+
Aerobic spore-forming rods (chiefly *B. subtilis, B. mycoides*, occasionally *B. teres*)	–	+	+ + +	–	+	+ + +	–	+	+ + +
Miscellaneous:									
Alcaligenes sps. (*A. metalcaligenes, A. faecalis*)	–	(+)	+	–	(+)	+	–	(+)	(+)
Flavobacterium (*F. lactis, F. helvolum, F. radiatum*)	–	–	(+)	–	–	(+)	–	–	(+)
Propionobacterium (*P. raffinosaceum*)	–	–	(+)	–	–	–	–	–	–
Achromobacteria (*A. candicans, A. lacticum*)	–	(+)	+	–	–	+	–	–	–

Key: + + + +, present in considerable numbers in all samples; + + +, present in lesser numbers in all samples; + +, present in moderate numbers in all samples; +, present in few numbers in all samples; (+), present in few numbers in occasional samples; –, absent.

process the growth of the surviving bacteria is generally retarded for a varying period of time, after which a considerable increase in numbers takes place; whereas H.T.S.T. pasteurized milk samples contain greater numbers of survivors which grow progressively as ageing proceeds. This peculiar feature of the L.T. 'Holder' pasteurized milk may be due to one or more of the following reasons:

(i) The destruction of accessory factors normally present in raw milk, which are necessary for the growth of some or all of the organisms which are heat-resistant.

(ii) The production of some inhibitory substance, which retards the growth of some or all survivors, but which ceases to have effect once the organisms have become acclimatized to it.

(iii) An injury sustained by the bacterial cells which interferes with their metabolism, but which after a certain time is restored, enabling the organisms to multiply normally.

This phenomenon in L.T. 'Holder' pasteurized milk samples has apparently escaped detailed attention by other workers, although references have been made to delayed germination in cultures which have been subjected to heat by Koch (44), Eijkman (45) Allen (46), Eckelman (47), Burke (48) and Abd-El-Malek & Gibson (49). On the other hand, some workers state that exposure to heat stimulates the growth of certain micro-organisms (Christian (50), Evans & Curran (51)).

The present investigation justifies the opinion that the process of L.T. 'Holder' pasteurization interferes (for a variable period of time) with the normal process of multiplication of a proportion of the organisms which survive. The effect also seems to be associated with an increase in the rate of deterioration during the later stages of ageing samples at 18° C.

Finally, numerical differences between the colony counts of L.T. 'Holder' and H.T.S.T. pasteurized milk samples are more pronounced in fresh than in aged samples. The corresponding correlation coefficients at successive intervals of time during ageing suggest that the numbers and types of the dominant peptonizing species which ultimately cause curdling are influenced by the sequence of changes which take place in the early stages of deterioration; these are themselves a result of the time/temperature effects of the two forms of heat treatment.

(2) *Percentage destruction of bacteria*

The higher percentage of survivors in the H.T.S.T. pasteurized milk samples appears to indicate that this method of pasteurization is less efficient than the L.T. 'Holder' system from a bactericidal point of view. This may or may not be true, since it is doubtful whether the plate counts of the fresh L.T. 'Holder' pasteurized milk samples were a reliable index of the numbers of micro-organisms actually present.

(3) *Coliform tests*

The number of fresh or aged pasteurized milk samples which yielded acid and gas reactions in one or two, and in one instance three out of five, MacConkey broth tubes, at any or all the three temperatures of incubation, can be summarized as follows:

Series	No. of exps.	No. of samples giving false positive reactions		% (of total examined)	
		L.T.	H.T.	L.T.	H.T.
A	36	13	8	41·7	22·2
B	14	2	1	14·3	7·1
Total	50	15	9	30·0	18·0

The single example of a false presumptive positive result referred to above, occurred in one H.T.S.T. sample in the first series of experiments. In some subcultures in MacConkey broth from apparent positive tubes a long-chained *Streptococcus* which formed acid and gas within the space of 1 or 2 hr. when incubated at 44° C. was noted, but all attempts to isolate coliforms failed.

The results disclosed a tendency for false positives to occur in the same fresh and aged samples at all three incubation temperatures. To some extent they also occured in both the L.T. 'Holder' and H.T.S.T. pasteurized milk samples obtained from the same raw milk. This suggests the presence of certain heat-resistant organisms, which have the ability to grow over a wide range of incubation temperatures and produce acid and gas in MacConkey broth.

The absence of coliforms in all pasteurized milk samples examined cannot be regarded as proof of the non-existence of heat-resistant species. It does, however, strengthen the opinion of other workers, notably Crossley (52), Vernon & Walker (53), Yale (18) and Wilson (54) that there is a low incidence of heat-resistant coliforms in efficiently produced L.T. 'Holder' and H.T.S.T. pasteurized milk.

(4) *Keeping quality*

The variable reduction times of the methylene-blue and resazurin tests carried out on the two series of fresh pasteurized samples is evidently caused by differences in reducing intensity in the three 10 ml. test portions, and can only be explained by an unequal distribution of organisms in the tubes when they were prepared. As it is known that the flora of both the fresh L.T. 'Holder' and H.T.S.T. pasteurized milk samples consisted mainly of acid-producing organisms, and only few peptonizing types, it is conceivable that 10 ml. quantities would contain proportionate numbers of the former group, but variable numbers of the latter. Also, since the peptonizing group become dominant on ageing and ultimately caused curdling, the actual numbers and species present in the small samples when fresh might be expected to bear some relationship to those present after incubation at 18° C. for 24–48 hr. or even longer. Thus the reducing capacities of small samples might be expected to show variation, dependent on the numbers and types of a minority group in the fresh samples but which later become predominant in the same aged samples and are the eventual cause of deterioration.

Confirmatory evidence of the poor reproducibility of dye reduction tests has been noted by Watson, Bessell, Goldschmidt & Davis (55), who found that the disparity between replicates increased as reduction times increased, and that various methods of mixing in the tubes had little if any effect on reproducibility.

Similar reduction tests carried out on the same pasteurized milk samples after ageing them for 45½ hr. showed good agreement between replicates. This appears to justify the view that the reproducibility of dye reduction tests increases as reduction times decrease, and indirectly leads to the assumption that the bacterial homogeneity of aged samples is much better than that of fresh samples.

Despite the high correlation coefficients between the dye reduction tests at 37, 30 and 22° C. on the 45½ hr. aged pasteurized milk samples, and between the plate counts at the same incubation temperatures of the fresh and aged pasteurized milk samples, no relationship existed between corresponding dye reduction tests and plate counts.

The experimental results show that the keeping quality of pasteurized milk can be

assessed by either reduction tests or alcohol tests provided the C.O.B. test is accepted as the reference standard of this quality. The higher correlation coefficients between alcohol and C.O.B. tests than between reduction tests and C.O.B. tests, affords an index of the relative values of the two tests for this purpose. In this series of experiments, it would appear that times to the end-points of all three tests are determined mainly by the activity of peptonizing organisms, which become increasingly predominant as samples are aged; and that each test in itself is a measure of the intensity effect. The study of the bacterial flora of samples at successive intervals of 24 hr. and the acidity of aged samples when reduction occurred and when positive reactions were given to the alcohol and C.O.B. tests, have indicated that a mixed flora containing a high proportion of peptonizing organisms is superseded by one in which this group becomes completely predominant as deterioration takes place. As end-point readings of the three tests occur successively, the correlation coefficients between the methylene-blue reduction tests and C.O.B. tests would be expected to be less close than between the alcohol and C.O.B. tests.

As reduction tests at 37, 30 and 22° C. on the pasteurized milk samples aged for 45½ hr. at 18° C. afforded a reasonable indication of keeping quality, it seems justifiable to assume that similar tests carried out after ageing for longer periods might further improve the relationship between these tests and C.O.B. tests.

Comparison has shown that the H.T.S.T. samples remained sweet for a slightly longer period of time than the L.T. 'Holder' samples. This difference has been explained in terms of the different effects produced by the two forms of heat treatment which not only affected the rates of multiplication of the surviving organisms during ageing, but also resulted in a higher proportion of peptonizing organisms being present in the L.T. 'Holder' samples than in the corresponding H.T.S.T. samples. Thus, although there was a similarity between the processes of deterioration in the two series of samples, the rates at which they occurred were dissimilar. In the L.T. 'Holder' samples maximum growth of organisms took place during the 24–48 hr. ageing period, so much so that the flora of 48 hr. old samples consisted mainly of peptonizing species. In the H.T.S.T. samples the proportion of peptonizers to total organisms was lower at 48 hr., indicating that the process of deterioration was taking place more slowly.

Ageing of the raw milk at 40–50° F. (4·4–10·0° C.) reduced the keeping quality of the corresponding pasteurized milk samples; this was attributed to the temperature of storage permitting the growth of heat-labile peptonizing organisms. This difference in the numbers of peptonizers in the pasteurized milk samples prepared from the fresh and aged raw milk, was reflected in marked differences in the bacterial group proportions after the samples had been aged for 48 hr. at 18° C., and this had the effect of causing a reduction in keeping quality.

Existing literature on the keeping quality of pasteurized milk is confusing owing to the different ways in which this property has been estimated. The findings herein reported are mainly illustrative of the differences between milks pasteurized by the L.T. 'Holder' and H.T.S.T. methods.

(5) *Bacterial flora of pasteurized milk samples*

The experimental results in this work have suggested that acid-producing organisms constituted the major flora of pasteurized milk samples when fresh. It has also been shown that the flora changes when samples are allowed to deteriorate at 18° C., so that

peptonizing organisms supersede acidifiers and are the eventual cause of curdling. The changes in bacterial group proportions during the first 48 hr. incubation at 18° C. support the findings of Rowlands & Provan (56) on samples of 'in-bottle' pasteurized milk stored at 15·5° C., but the ability of acid-forming organisms (chiefly *Streptococcus lactis*) to become predominant after this time, reported by Rowlands & Provan (56), was not in accordance with the findings in this work.

The bacterial species found to survive pasteurization in these experiments substantiate the opinion of Hileman, Leber & Speck (57), that H.T.S.T. samples contain a greater diversity of species than L.T. 'Holder' samples. Of the many species noted by these authors in pasteurized milk only *Streptococcus thermophilus*, *Microbacterium albus* and *M. flavus* were unidentified in this work. Hileman *et al.* (57) also found a much higher percentage survival of alkali-forming species in H.T.S.T. pasteurized milk samples (30·8%) than in corresponding L.T. 'Holder' samples (4·1%), but these results have not so far been corroborated.

The high incidence of Microbacteria in isolations from 30 and 22° C. plates of freshly pasteurized milk samples has been noted, and confirms the report of Gibson & Abd-El-Malek (58) and Abd-El-Malek (59) that this group constitutes the predominant microflora of laboratory pasteurized milk plates incubated at 30° C.

The frequency with which *Streptococcus plantarum* was isolated from the 37 and 30° C. plates is perhaps of special significance, as other workers have failed to notice its occurrence to this extent in pasteurized milk. Its survival is not, however, surprising in view of the work of Orla-Jensen (60), who showed that four cultures in casein peptone dextrose agar were able to withstand heating to 60° C. for 15 min., and two of the same cultures tolerated exposure at 70° C. for a similar period.

The question of survival of *Str. lactis* appears to be a matter of considerable interest, owing to the report of Rowlands & Provan (56) that a few cells were able to survive pasteurization by the 'in-bottle' method and eventually caused the souring of samples aged at 15·5° C. The absence of this species in pasteurized milk samples in either this experimental work or that of Hileman *et al.* (57) has been noted. A general conclusion that the process of deterioration in pasteurized milk is mainly due to the activity of this species appears to be of doubtful validity in view of the recent work by Abd-El-Malek & Gibson (61), who have shown that erratic growth took place when certain strains of *Str. lactis* in milk were exposed to temperatures of 60° C. for 30 min. and growth was still less frequent after heating at 63° C.

SUMMARY

1. Examinations have been made of milk pasteurized by the L.T. 'Holder' and H.T.S.T. processes using the same original bulk raw milk. In the preparation of samples care was taken to eliminate the possibility of post-pasteurization contamination. It was hoped that any differences found would be due solely to the effects of the pasteurizing processes above.

2. Samples were examined by (*a*) colony counts and coliform tests when fresh and after ageing at 18° C. for 24 and 48 hr., (*b*) methylene-blue and resazurin reduction tests when fresh and after ageing at 18° C. for 45½ hr., (*c*) keeping quality at 18° C. to end-point of 68% alcohol and clot-on-boiling tests. All bacteriological and dye reduction tests were carried out at incubation temperatures of 37, 30 and 22° C.

3. In seven trials the experiments were repeated after storage of the original raw milk for 24 hr. at 40–50° F. (4·4–10·0° C.).

4. Some detailed examinations were made of the bacterial floras of the raw and corresponding pasteurized milks, both when fresh and after ageing.

5. The percentage survival of organisms was greater in H.T.S.T. than in 'Holder' pasteurized milk, but the keeping quality of 'Holder' pasteurized milk was inferior to that of H.T.S.T. pasteurized milk.

6. Differences between the keeping quality of 'Holder' and H.T.S.T. pasteurized milk suggested fundamental differences in the effects of the two time/temperature combinations, which influenced the behaviour of samples aged at 18° C. Peptonizing organisms appeared to be the major cause of deterioration of pasteurized milk in which post-pasteurization contamination was prevented.

7. H.T.S.T. pasteurized milk contained the more varied flora, and bacterial multiplication proceeded steadily during ageing. In 'Holder' pasteurized milk bacterial growth appeared to be retarded during the first 24 hr. at 18° C., but thereafter peptonizing organisms developed with great rapidity, with significant effect upon keeping quality.

8. Correlation between clot-on-boiling and 68% alcohol tests was good. Methylene-blue reduction times at 37° C. of aged samples were a useful guide to keeping quality, but similar tests on fresh pasteurized milks were of little value.

9. Ageing of raw milk at 40–50° F. (4·4–10·0° C.) increased the thermoduric peptonizing flora and reduced the keeping quality of the corresponding pasteurized milk. Nevertheless, bacteriological and dye reduction test results of raw milk bore little relation to the keeping quality of the pasteurized milk.

10. Plate-colony counts of pasteurized milk gave no indication of its keeping quality. Their value seemed limited to an indication of the general standard of production and handling of the milk prior to pasteurization.

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REFERENCES

(1) Ministry of Health (1941). Circ. No. 2423. Milk (Special Designations) Regulations. H.M.S.O.
(2) North, C. E. & Park, H. (1929). *Amer. J. Hyg.* **7**, 147.
(3) Provan, A. L. & Rowlands, A. (1939). *Abstr. Proc. Soc. agric. Bact.* p. 15.
(4) Clegg, L. F. L. & Lomax, K. L. (1946). *Dairy Industr.* **11**, 488.
(5) Davis, J. G. & Watson, D. W. (1946). *Proc. Soc. appl. Bact.* p. 81.
(6) McKenzie, D. A. & Booth, C. E. (1945). *Proc. Soc. appl. Bact.* p. 31.
(7) McKenzie, D. A. (1947). *Dairy Industr.* **12**, 764.
(8) Thomas, S. B., Griffiths, D. G., Morgan, K. J. & Lewis, T. J. (1948). *Dairy Industr.* **13**, 443.
(9) Thomas, S. B. & Clegg, L. F. L. (1948). *Dairyman*, **65**, 101.
(10) Mattick, A. T. R. & Hiscox, E. R. (1937). *11th Worlds' Dairy Congr. Rep.* **3**, 344.
(11) Reid, A. W. (1946). *Dairy Industr.* **11**, 740.
(12) Egdell, J. W. & Bird, E. R. (1949). *J. Soc. Dairy Tech.* **2**, 144.
(13) United States Public Health Service (1939). *Publ. Hlth Bull.* no. 220.
(14) Dahlberg, A. C. (1932). *Tech. Bull. N.Y. St. agric. Exp. Sta.* no. 203.

(15) HOLLAND, R. S. & DAHLBERG, A. C. (1940). *Tech. Bull. N.Y. St. agric. Exp. Sta.* no. 254.
(16) HOLMQUIST, C. A. (1932). *Ann. Rep. int. Ass. Dairy Insp., Wash.*, **20**, 79.
(17) MATTICK, A. T. R. & HISCOX, E. R. (1939). *Med. Offr*, **61**, 177.
(18) YALE, M. W. (1933). *Tech. Bull. N.Y. St. agric. Exp. Sta.* no. 207.
(19) QUINN, J. D. & BURGWALD, L. H. (1933). *Milk Pl. Mthly*, **22**, 26.
(20) WORKMAN, T. W. (1941). *Ass. Bull. int. Ass. Milk Dlrs*, **33**, 585.
(21) PARFITT, E. H. (1940). 13*th Ann. Rep. N.Y. Ass. Dairy Insp., Wash.*, p. 195.
(22) KREUGER, P. F. (1938). *J. Milk Tech.* **1**, 29.
(23) WEINRICH, C. F. (1946). *Spec. Bull. Mich. St. agric. Exp. Sta.* no. 335, p. 83.
(24) FABIAN, F. W. (1942). *J. Milk Tech.* **5**, 237.
(25) MILLENKY, A. & BRUECKNER, H. J. (1941). *Bull. Cornell agric. Exp. Sta.* no. 754.
(26) HILEMAN, J. L. & LEBER, H. (1941). *J. Milk Tech.* **4**, 128.
(27) LIPMAN, L. D., FRANDSEN, J. H. & LINDQUIST, H. G. (9141). *Bull. Mass. agric. Exp. Sta.* no. 378, p. 54.
(28) BURGWALD, L. H. (1946). *Spec. Bull. Mich. St. Coll. agric. Exp. Sta.* no. 335, p. 10.
(29) DUMMETT, G. A. & MONGAB, J. L. (1944). *Dairy Industr.* **9**, 264.
(30) DAVIS, J. G. & SADEK, G. M. (1942). *Milk Ind.* **22** (10), 33.
(31) WILSON, G. S. (1935). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 206, p. 215.
(32) DAVIS, J. G. (1939). *Food Manuf.* **14**, 196.
(33) MINISTRY OF AGRICULTURE (1942). Technique for the Standard Resazurin Test. Form No. C. 158/TPY.
(34) MINISTRY OF HEALTH (1944). Statutory Rules and Orders no. 349. Heat Treated Milk (Prescribed Tests) Order. H.M.S.O.
(35) MINISTRY OF HEALTH (1937). Memo 139/Foods. H.M.S.O.
(36) ANDERSON, E. B. & WILSON, G. S. (1945). *J. Dairy. Res.* **14**, 21.
(37) NEAVE, F. K. (1939). *J. Dairy Res.* **10**, 474.
(38) FISHER, R. A. (1938). *Statistical Methods for Research Workers*, 7th ed. London: Oliver and Boyd.
(39) AMERICAN PUBLIC HEALTH ASSOCIATION (1925). *Standard Methods of Water Analysis*, 6th ed. New York: Amer. Publ. Hlth Ass.
(40) ROWLANDS, A. & PROVAN, A. L. (1941). *Abstr. Proc. Soc. agric. Bact.* p. 36.
(41) HARRISON, J. (1938). *Abstr. Proc. Soc. agric. Bact.* p. 12.
(42) BERGEY, D. H. *et al.* (1939). *Manual of Determinative Bacteriology*, 5th ed. London: Baillière, Tindall and Cox.
(43) HISCOX, E. R. (1943). *Abstr. Proc. Soc. agric. Bact.* p. 14.
(44) KOCH, R. (1881). *Mitt. Reichsgesundh. Amt.* **1**, 234.
(45) EIJKMAN, C. (1908). *Biochem. Z.* **11**, 12.
(46) ALLEN, P. W. (1923). *J. Bact.* **8**, 555.
(47) ECKELMANN, E. (1917). *Zbl. Bakt.* Abt. II, **48**, 140.
(48) BURKE, G. S. (1923). *J. infect. Dis.* **33**, 274.
(49) ABD-EL-MALEK, Y. & GIBSON, T. (1948). *J. Dairy Res.* **15**, 249.
(50) CHRISTIAN, M. L. (1931). *J. Dairy Res.* **3**, 113.
(51) EVANS, P. W. & CURRAN, H. R. (1943). *J. Bact.* **46**, 513.
(52) CROSSLEY, E. L. (1946). *J. Dairy Res.* **14**, 233.
(53) VERNON, T. R. & WALKER, L. J. (1936). *Soc. agric. Bact. Ann. Conf. Paper*, no. 7.
(54) WILSON, G. S. (1935). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 206, p. 175.
(55) WATSON, D. W., BESSELL, C. J., GOLDSCHMIDT, E. & DAVIS, J. G. (1946). *Proc. Soc. appl. Bact.* **1**, 56.
(56) ROWLANDS, A. & PROVAN, A. L. (1941). *Abstr. Proc. Soc. agric. Bact.* p. 29.
(57) HILEMAN, J. L., LEBER, H. & SPECK, M. L. (1941). *J. Dairy Sci.* **24**, 305.
(58) GIBSON, T. & ABD-EL-MALEK, Y. (1940). *Abstr. Proc. Soc. agric. Bact.* p. 19.
(59) ABD-EL-MALEK, Y. (1943). *Abstr. Proc. Soc. agric. Bact.* p. 23.
(60) ORLA-JENSEN, S. (1942). *The Lactic Acid Bacteria*, 2nd ed. Copenhagen: Munksgaard.
(61) ABD-EL-MALEK, Y. & GIBSON, T. (1948). *J. Dairy Res.* **15**, 233.

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427. THE ACID PHOSPHATASE OF COWS' MILK

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(With 5 Figures)

I. SOME PROPERTIES OF THE ENZYME

The presence in cows' milk of an acid phosphatase has not been fully investigated, although Huggins & Talalay (1), using phenolphthalein phosphate as a substrate, found hydrolysis at an acid pH. Further investigations were not reported. Giri (2), investigating the phosphatases in human milk, found an acid phosphatase with an optimum peak of activity at pH 5·1, and later, Vittu (3), also working on human milk, suggested that two acid phosphatases existed and were active between pH 3·6 and 5·9.

Acid phosphatases have been known since 1924, when Martland, Hansman & Robison (4) investigated the phosphatase activity of blood. Since then acid phosphatases have been investigated in a number of animal organs, particularly in the male sex organs.

Results from the investigations described in this paper have shown that an acid phosphatase exists in cows' milk, and that the enzyme possesses certain unique properties.

METHODS

Bulk milk from the cows in the Institute herd was used in the experiments, except when otherwise stated.

Gutman & Gutman (5) have adapted the method of King & Armstrong (6) for the estimation of alkaline phosphatase to estimating acid phosphatase in blood serum. This method, with certain modifications, has been found to be satisfactory for estimating acid phosphatase activity in cows' milk. The modifications include the use of the succinic acid-borax buffer of Kolthoff (7), and a shorter period of incubation. Higher values for enzyme activity were observed using this buffer, than when the citrate buffer, used by Gutman & Gutman (5) in their method, was employed. The unit of enzyme activity was as defined by Gutman & Gutman (5), namely, the quantity of enzyme which causes the liberation of 1 mg. phenol in 1 hr. under standard conditions of hydrolysis.

A photoelectric absorptiometer, with a Dufay spectrum filter with maximum transmission near 690 mμ, was used. A calibration curve and factor were obtained by using known amounts of phenol.

The procedure for estimating the enzymic activity in milk was as follows: to 10 ml. of substrate solution (0·005 M-disodium phenylphosphate and buffered at pH 3·95 at 18° C.), 0·5 ml. of milk was added and the whole mixture maintained at 37° C. in a water-bath for 1 hr. To this mixture, 4·5 ml. of Folin and Ciocalteu's phenol reagent diluted 1:2 with 5% (w/v) sodium hexametaphosphate, was then added. After 10 min. the mixture was filtered through a Whatman no. 42 filter-paper and 2·5 ml. of 20% (w/v) Na_2CO_3 added to 10 ml. of the filtrate. This mixture was then maintained at 37° C. for 30 min., and the resulting colour was read in the photoelectric absorptiometer. Controls were incubated without milk, the milk being added immediately after the addition of the phenol reagent.

All pH measurements were made by a direct-reading Cambridge pH meter using a glass electrode.

RESULTS

The effect of pH *on the activity of the acid phosphatase*

The effect of pH on the enzyme may be seen in Fig. 1, which clearly shows a peak of activity at pH 4·0 (using a citrate buffer). Similar results have been obtained with an acetate buffer and also with a succinic acid-borax buffer.

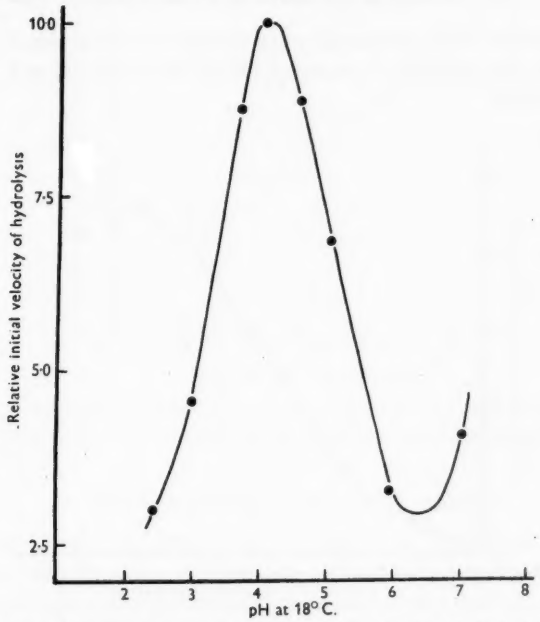

Fig. 1. Activity-pH curves for acid phosphatase of cows' milk. Hydrolysis at 37° C. in the presence of 0·005 M-substrate. Reaction time 3 hr.

Table 1. *Hydrolysis of* 0·005-M *phenylphosphate by acid phosphatase at* 37° C.*

Time in min.	Amount hydrolysed (%)	$k \times 10^{-4}$
15	0·07	0·46
30	0·13	0·43
45	0·19	0·42
60	0·26	0·43
90	0·35	0·39
120	0·43	0·36
150	0·51	0·35
180	0·57	0·32

* pH of incubation mixture—4·05 at 20° C.

The rate of hydrolysis by acid phosphatase

A linear reaction for the hydrolysis of disodium phenylphosphate by acid phosphatase was observed up to 60 min. after the start of the reaction. Table 1 gives the values of the reaction constant as calculated from the equation

$$k = \frac{2{\cdot}303}{t} \log \frac{a}{a-x},$$

where t is the time of hydrolysis, a is the initial concentration of the substrate and x the amount hydrolysed in the time t.

The determination of the Michaelis constant

In Fig. 2 the 'Michaelis curve' of the relation between substrate concentration and initial rate of hydrolysis is shown. The method of Folley & Kay (8) is used in presenting the data. The Michaelis constant is calculated from the equation $\frac{v}{V}=\frac{s}{s+K_m}$, where s is the molar concentration of the substrate, v the velocity of hydrolysis, K_m the dissociation constant of the enzyme-substrate compound (Michaelis constant) and V is the limiting velocity. K_m is 0·0032.

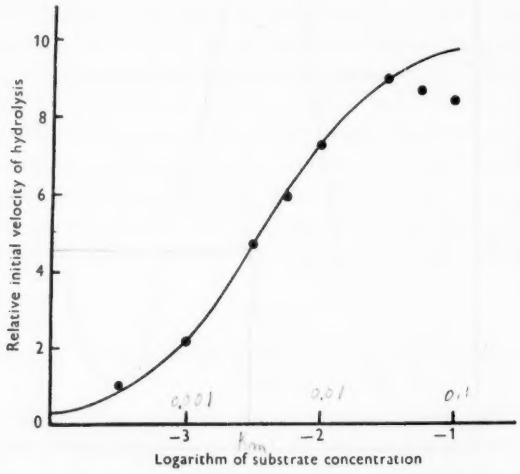

Fig. 2. Relation between substrate concentration and enzyme activity for acid phosphatase in cows' milk. Hydrolysis at 37° C. Reaction time 1 hr. at pH 4·1.

At substrate concentrations exceeding 0·032 M, the effect was consistent with the theory of Haldane (9), in that inhibition of hydrolysis was occurring. The values for the constants K_1 and K_2, in the Haldane equation

$$v=\frac{K}{1+K_1/s+s/K_2},$$

are 0·004 and 0·4 respectively.

The effect of temperature on rate of hydrolysis by acid phosphatase

A straight-line relationship between the velocity of hydrolysis and temperature was observed between 14 and 50° C. (see Fig. 3). Between 20 and 50° C. the temperature coefficients were:

20–30° C.	1·41
30–40° C.	1·29
40–50° C.	1·22

The energy of activation of the enzyme was calculated from the slope of the line obtained by plotting log K against $1/T$, obtained from the Arrhenius equation

$$\ln \frac{K_2}{K_1} = \frac{A}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right),$$

where K is the rate of hydrolysis and T is the absolute temperature. The value obtained was 5100 cal./mole.

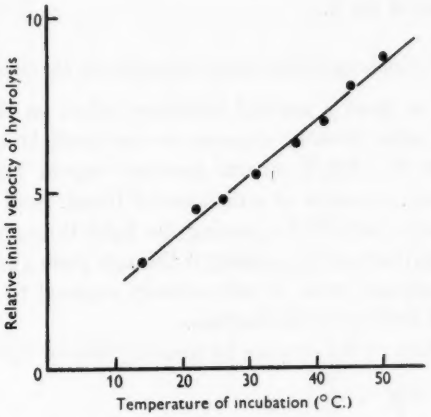

Fig. 3. Relation between temperature of hydrolysis and enzyme activity for acid phosphatase in cows' milk. Hydrolysis at pH 4·1 in the presence of 0·005 M-substrate. Reaction time 1 hr.

The thermolability of the acid phosphatase

The enzyme was found to be very stable towards heat. Table 2 shows the amount of destruction of the enzyme after various time-temperature combinations between 88·2 and 100° C. A time-lag, short but difficult to measure exactly, was experienced in heating the milk (0·5 ml. milk was pipetted into a thin-walled test-tube surrounded by a relatively large volume of water at the required temperature, held, removed, and cooled by the addition of 10 ml. buffer substrate), but the results show clearly that the enzyme is relatively thermostable by comparison with most enzymes occurring in body fluids. Under normal 'Holder' pasteurization conditions (62·8° C. for 30 min.), milk retained about 80–90% of the original enzyme content.

Table 2. *Percentage destruction of acid phosphatase by heat*

Temp. (° C.)	Time of heating in min.										
	½	1	2	3	4	5	6	9	12	15	30
100	93	100	—	—	—	—	—	—	—	—	—
99·4	67	97	100	—	—	—	—	—	—	—	—
98·9	39	85	99	100	—	—	—	—	—	—	—
98·4	—	74	97	99	100	—	—	—	—	—	—
95·9	—	63	92	98	99	100	—	—	—	—	—
93·3	—	—	—	88	—	—	98	99	100	—	—
90·8	—	—	—	78	—	—	93	96	98	100	—
88·2	—	—	—	62	—	—	83	89	94	94	100

The action of acid phosphatase on phosphoric esters

The hydrolysis of the esters of α- and β-glycerophosphoric acid and of *p*-nitrophenylphosphoric acid was investigated. Liberated phosphorus was estimated by the method of Sumner (10) with minor modifications. *p*-Nitrophenylphosphate was easily hydrolysed at pH 4·0, 1·5 times as fast as phenylphosphate.

Hydrolysis of the esters of glycerophosphoric acid was slow; that of the α-isomer being slightly faster than that of the β.

The effect of visible and ultra-violet radiation on the acid phosphatase

Sunlight was found to have a marked inhibitory effect on the enzyme, up to 50% inactivation occurring after 60 min. exposure to the sunlight of a bright spring day. Radiation from an 800 W., 220 V. quartz mercury vapour lamp caused up to 80% inactivation after 30 min. exposure at a distance of 15 cm. from the lamp. The effect of such radiation was much reduced by passing the light through a Woods filter (transmission peak at 395 mμ.) but not by passing it through plate glass.

The addition of unexposed milk to milk already exposed to such radiation did not bring back the original activity of the enzyme.

The rate of inactivation of the enzyme by mercury vapour light is given in Fig. 4.

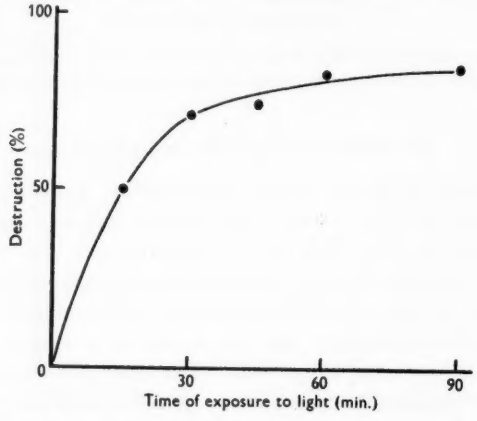

Fig. 4. Relation between time of exposure to mercury vapour light and enzyme activity for acid phosphatase in cows' milk. Hydrolysis at pH 4·05 in the presence of 0·005 M-substrate. Reaction time 1 hr. at 37° C.

The effect of various substances on the activity of the acid phosphatase

The effect of a number of substances on the enzyme has been studied. These are summarized in Table 3 together with the limits of concentration causing the effect.

The enzyme was adsorbed by Fuller's earth and kaolin, while aluminium hydroxide appeared slightly to activate the enzyme.

The stability of the acid phosphatase in cows' milk

The enzyme maintained its activity in cows' milk at 3° C. for 48 hr., while at 17° C. the enzyme activity was reduced about 40% (see Fig. 5).

Table 3. *The effect of various substances on the activity of acid phosphatase* (0·005-M *phenylphosphate*; 37° C.; pH 4·1 *at* 20° C.)

	Molar concentration $\times 10^{-3}$ during hydrolysis	Relative hydrolysis
Sodium fluoride	0	1·00
	2·83	0·25
	5·66	0·18
	11·32	0·04
	22·64	0
Magnesium chloride	0	1·00
	0·227	1·07
	1·132	1·02
	4·55	1·02
	11·32	0·94
Manganese sulphate	0	1·0
	0·476	2·2
	0·715	3·0
	0·952	4·0
Chloroform	0	1·0
	Saturated solution	0·9
Formaldehyde	0	1·0
	1% (v/v) formalin solution	0·8
Hydrogen-ion concentration (pH)*	4·85	0·82
	5·65	0·97
	6·76	1·00
	8·00	0·94
	10·05	0·54

* Milks (in the absence of buffer substrate solution) were titrated to various pH values with 0·1 N-NaOH or HCl and maintained at that pH for 1 hr. at 37° C. The titrated milks were then neutralized to pH 6·8 and the activity of the enzyme measured after this treatment.

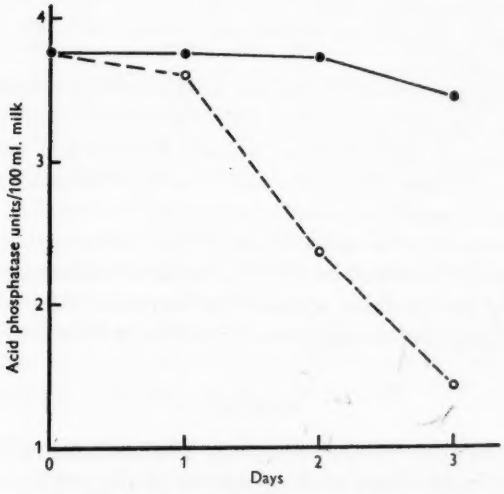

Fig. 5. Relation between the stability of the acid phosphatase in cows' milk and the age and temperature. Kept at 3° C. (black dots) and 18° C. (circles). Hydrolysis at pH 4·08 in the presence of 0·005 M-substrate. Reaction time 1 hr. at 37° C.

DISCUSSION

That the enzyme is capable of hydrolysing phosphoric esters at pH 4·0 is evident, though the hydrolysis of the glycerophosphoric esters was very slow. Results obtained after a lengthy period of incubation are not those to which much significance can be attached. However, a slightly greater hydrolysis of the α-isomer was apparent, and it is interesting to note that Roche, Thoai & Baudin (11) found an erythrocyte phosphatase active at pH 4·1, with a preferential rate of hydrolysis on α-glycerophosphate. The classification of the phosphatases made by Folley & Kay (12) includes those with optima at an acid pH (pH 3–4 and 4·5–5), but these have a greater affinity for β-glycerophosphate.

A zero order of reaction was evident up to 60 min. hydrolysis, after which time a decrease in hydrolysis was observed. This might be attributed to enzyme destruction or to the effects of pH.

According to Sizer (13) an enzyme may be regarded as being an efficient catalyst if it possesses a low energy of activation. The value obtained for acid phosphatase is 5100 cal./mole, which may be regarded as low; the majority of enzymes whose energies of activation are listed by Sizer have a value exceeding 10,000 cal./mole. By this theory, acid phosphatase may be regarded as a relatively efficient enzyme.

The decrease in the value of the temperature coefficients with an increase in temperature has already been observed by Haldane (9) to be a characteristic of hydrolytic enzymes.

One of the most interesting properties of acid phosphatase is its stability towards heat. The normal conditions of pasteurization bring about no more than a 10–20% inactivation. The well-known alkaline phosphatase, on the contrary, is almost totally destroyed at the time-temperature combination required for the pasteurization of milk. It is interesting to note that Riisfeldt (14), using his modification of the Gutman & Gutman method (5), stated that pasteurized milk did not contain acid phosphatase. He did not report any investigations on raw milk.

A further interesting property of the enzyme is its instability towards visible radiation. The effect of light on enzymes has not been widely investigated, but recently Kay (15) has shown that milk lipase is quite sensitive towards sunlight and ultra-violet radiation. Experimental results indicate that in the case of acid phosphatase of milk, the visible part of the spectrum is mainly responsible for the inactivation. The instability towards light is in marked contrast to the enzyme's exceptional thermostability.

The enzyme is strongly inhibited by fluoride, but the stimulating effect of magnesium, a notable activator of phosphatases, is small. The enzyme is inhibited by chloroform and formaldehyde. It is clear that the enzyme is very different from the alkaline phosphatase of cows' milk.

SUMMARY

An enzyme in milk, capable of hydrolysing phenylphosphate and other phosphoric esters at pH 4·0, has been found. Some of the properties of the enzyme are described, particularly the effect of heat and light on it.

REFERENCES

(1) HUGGINS, H. & TALALAY, P. (1948). *J. biol. Chem.* **159**, 399.
(2) GIRI, K. V. (1936). *Hoppe-Seyl. Z.* **243**, 57.
(3) VITTU, C. (1946). *C.R. Soc. Biol., Paris*, **140**, 225.
(4) MARTLAND, M., HANSMAN, F. S. & ROBISON, R. (1924). *Biochem. J.* **18**, 1152.
(5) GUTMAN, E. B. & GUTMAN, A. B. (1940). *J. biol. Chem.* **136**, 201.
(6) KING, E. J. & ARMSTRONG, A. R. (1934). *Canad. med. Ass. J.* **31**, 376.
(7) KOLTHOFF, I. M. (1925). *J. biol. Chem.* **63**, 135.
(8) FOLLEY, S. J. & KAY, H. D. (1935). *Biochem. J.* **29**, 1837.
(9) HALDANE, J. B. S. (1930). *Enzymes.* London: Longmans, Green and Co.
(10) SUMNER, J. B. (1944). *Science*, **100**, 413.
(11) ROCHE, J., THOAI, N-v. & BAUDIN, J. (1942). *C.R. Acad. Sci., Paris*, **215**, 386.
(12) FOLLEY, S. J. & KAY, H. D. (1936). *Ergebn. Enzymforsch.* **5**, 159.
(13) SIZER, I. W. (1943). *Advances in Enzymology*, **3**, 35.
(14) RIISFELDT, O. (1946). *Acta path. microbiol. scand.* Suppl. p. 58.
(15) KAY, H. D. (1946). *Nature, Lond.*, **157**, 511.

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II. VARIATION IN THE ACID PHOSPHATASE CONTENT OF MILK FROM NORMAL COWS AND DURING THE PROGRESS OF LACTATION

(With 4 Figures)

From Part I of this paper it is clear that an acid phosphatase exists in cows' milk and that this enzyme is capable of hydrolysing monoesters of phosphoric acid. The concentration of the enzyme in milk is low, being about a fortieth of the concentration of the alkaline phosphatase present. In the classification of the phosphomonoesterases made by Folley & Kay (2) the acid phosphatase of milk falls most nearly into group AII.

Folley & Kay (3) have shown that the alkaline phosphatase concentration of milk follows a definite pattern throughout the lactation period of the cow. It was thought very probable that the acid phosphatase concentration might also show a definite pattern in relation to the lactation period, and the data given in the present paper indicate such a relationship.

EXPERIMENTAL

The acid phosphatase levels of milks from fifty-eight cows in the Institute herd were estimated by the procedure employed by Mullen (1). These cows were in various stages of lactation between the fiftieth day following parturition and prior to the thirtieth day before drying off. The enzyme levels in a number of bulk milks were also estimated; the bulk milks contained milk from approximately thirty-five cows in all stages of lactation.

The milks from four Shorthorn cows belonging to the Institute herd were studied from parturition to the end of lactation. These cows were not isolated from the herd and underwent all the normal routine of feeding for the time of year and stage of lactation. Samples of milk were taken at the morning milking period during the progress of lactation. For the first 50–60 days samples were taken every day. Up to 80 days samples were taken three times a week, and up to 120 days once a week. For the remainder of the period,

samples were taken once every 2 weeks until the latter part of the lactation, when daily sampling was resumed. Milk acid phosphatase was estimated by the same method as employed above. The unit of acid phosphatase is that defined by Gutman & Gutman (4). It was observed *inter alia* that variation between morning and evening samples did not exceed that of daily variation.

RESULTS

Table 1 shows the range of acid phosphatase levels in individual milks and bulk milks.

Figs. 1–4 show graphically the concentration, in enzyme units per 100 ml. milk, throughout lactation, together with the weekly milk yield in lb.

The total yield of acid phosphatase at each morning milking was calculated; the enzyme yields throughout lactation are shown in Table 2.

Table 1. *The range of acid phosphatase levels of cows' milk*

	Units per 100 ml. milk					
	2	2–4	4–6	6–8	8–10	10
	Individual milks					
No. of samples	1	18	18	15	4	2
	Bulk milks					
No. of samples	0	7	30	33	9	3

Table 2. *The total yield of acid phosphatase in cows' milk at the morning milking*

Clara 11		Lily 7		Flora 70		Rosalie 14	
Days from parturition	Phosphatase units	Days from parturition	Phosphatase units	Days from parturition	Phosphatase units	Days from parturition	Phosphatase units
6	1455	6	1829	6	908	6	2400
7	—	7	1463	7	1058	7	2490
8	—	8	943	8	998	8	—
9	503	9	874	9	—	9	2044
10	602	10	886	10	—	10	2216
11	467	11	1355	11	645	11	—
12	300	12	768	12	852	12	1813
13	204	13	824	13	953	13	1627
14	343	14	621	14	869	14	1637
15	397	15	705	15	1072	15	—
16	454	16	714	16	841	16	1498
17	344	17	410	17	604	17	1571
18	308	18	688	18	932	18	1345
19	270	19	—	19	1471	19	705
20	214	20	1102	20	960	20	1258
21	297	21	1287	21	826	21	1611
22	218	22	—	22	746	22	—
23	320	23	878	23	900	23	1321
24	225	24	951	24	838	24	1323
25	232	25	1259	25	947	25	—
26	221	26	896	26	943	26	1544
27	254	27	808	27	841	27	1665
28	251	28	819	28	723	28	—
29	250	29	734	29	812	29	1440
30	249	30	774	30	959	30	1234
31	255	31	795	31	813	31	1261
32	265	32	812	32	778	32	1263
33	245	33	761	33	804	33	1039
34	275	34	740	34	758	34	1068
35	259	35	751	35	—	35	1062
36	413	36	963	36	844	36	909
37	216	37	647	37	755	37	838
38	220	38	626	38	883	38	1005
39	232	39	606	39	900	39	1067

Table 2 (*continued*)

Clara 11		Lily 7		Flora 70		Rosalie 14	
Days from parturition	Phosphatase units	Days from parturition	Phosphatase units	Days from parturition	Phosphatase units	Days from parturition	Phosphatase units
40	208	40	591	40	927	40	1072
41	320	41	556	41	799	41	1177
42	181	42	338	42	739	42	1082
43	286	43	501	43	805	43	1053
44	259	44	578	44	706	44	1025
45	252	45	503	45	705	45	880
46	218	46	623	46	786	46	792
47	209	47	496	47	697	47	771
48	235	48	530	48	731	48	931
49	203	49	443	49	688	49	1025
50	168	50	472	50	679	50	873
51	199	51	477	51	579	51	717
52	166	53	560	52	555	53	731
54	152	56	392	54	587	56	511
56	169	58	356	56	715	58	784
59	214	60	210	58	724	60	821
61	169	63	371	61	697	63	731
63	212	65	320	63	483	65	501
66	218	67	430	65	570	67	683
69	182	70	410	68	490	70	601
70	148	72	—	70	411	72	470
73	148	74	388	72	446	74	560
75	154	77	436	75	531	77	649
77	132	79	397	77	500	79	613
80	161	81	332	79	525	81	608
87	133	84	381	82	484	84	822
94	139	91	262	89	602	91	783
101	181	98	389	96	500	98	631
108	136	105	335	103	528	105	714
115	113	119	310	110	416	112	478
122	119	133	300	124	361	126	452
129	99	147	280	138	373	140	530
143	87	161	253	152	365	154	556
157	93	175	261	166	299	168	422
171	91	189	287	188	218	182	369
185	139	203	357	194	232	198	373
199	117	217	236	208	291	210	454
213	126	231	175	222	238	224	383
229	233	245	175	236	212	238	360
241	241	259	109	250	247	252	357
—	—	266	114	264	191	266	243
—	—	273	107	278	138	280	259
—	—	280	91	292	229	294	237
—	—	283	101	295	190	301	212
—	—	287	108	296	102	303	222
—	—	289	88	—	—	305	172
—	—	291	104	—	—	307	195
—	—	292	105	—	—	309	230
—	—	—	—	—	—	310	250
—	—	—	—	—	—	311	198
—	—	—	—	—	—	312	129
—	—	—	—	—	—	313	135
—	—	—	—	—	—	314	190
—	—	—	—	—	—	315	204
—	—	—	—	—	—	316	207
—	—	—	—	—	—	317	161
—	—	—	—	—	—	318	115
—	—	—	—	—	—	319	135
—	—	—	—	—	—	320	99
—	—	—	—	—	—	321	123
—	—	—	—	—	—	322	130
—	—	—	—	—	—	323	91
—	—	—	—	—	—	325	58
—	—	—	—	—	—	326	95
—	—	—	—	—	—	327	67

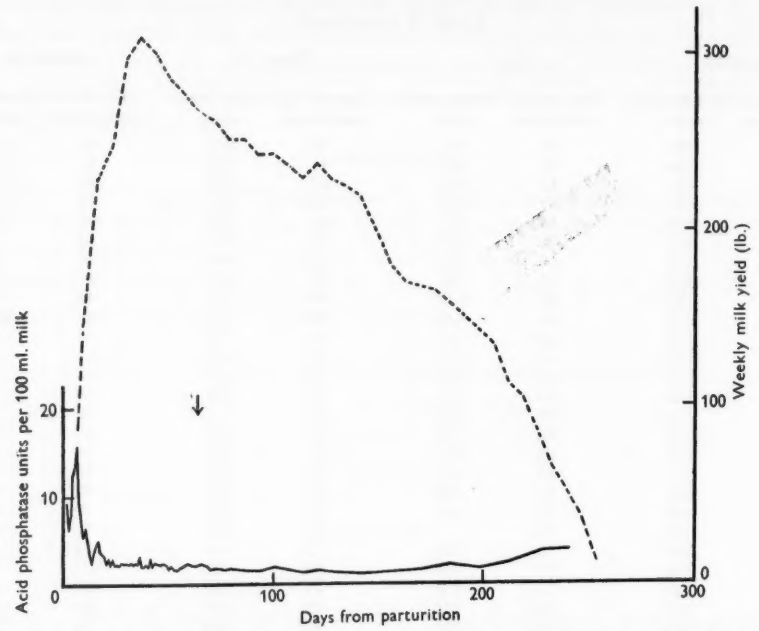

Fig. 1. Clara 11. The relation between the acid phosphatase concentration (full line), the weekly milk yield (dotted line) and the stage of lactation. Conception date indicated by arrow.

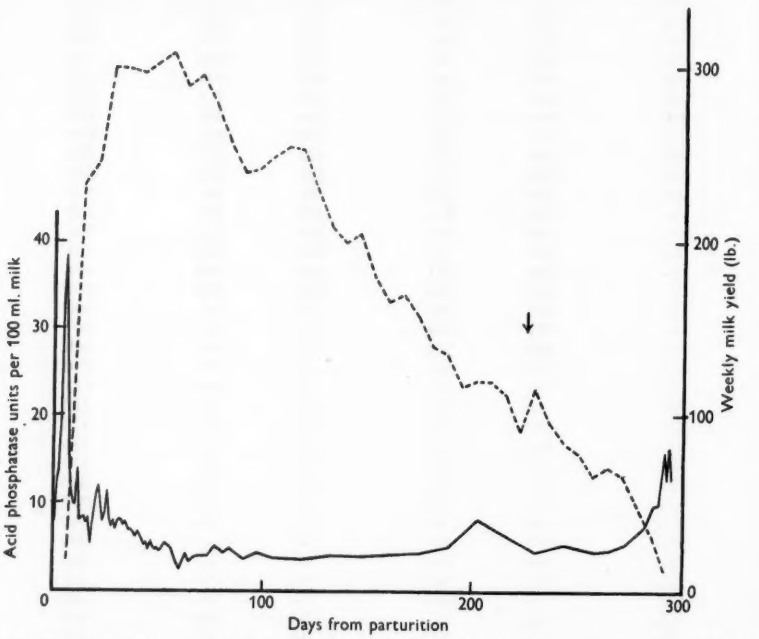

Fig. 2. Lily 7. The relation between the acid phosphatase concentration (full line), the weekly milk yield (dotted line) and the stage of lactation. Conception date indicated by arrow.

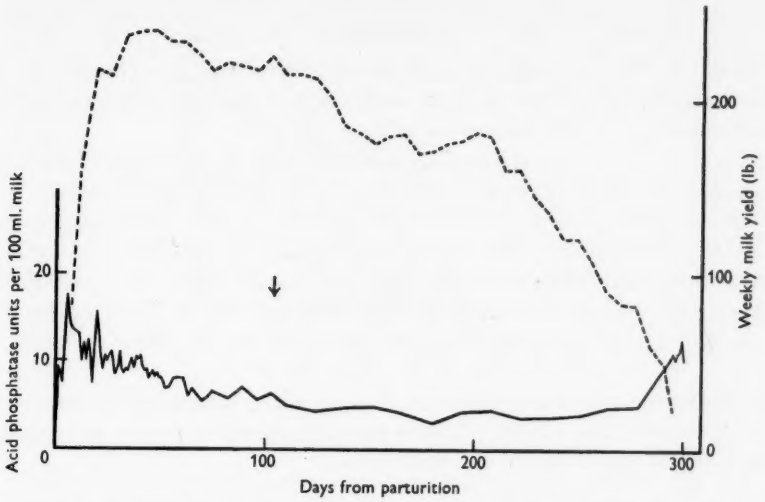

Fig. 3. Flora 70. The relation between the acid phosphatase concentration (full line), the weekly milk yield (dotted line) and the stage of lactation. Conception date indicated by arrow.

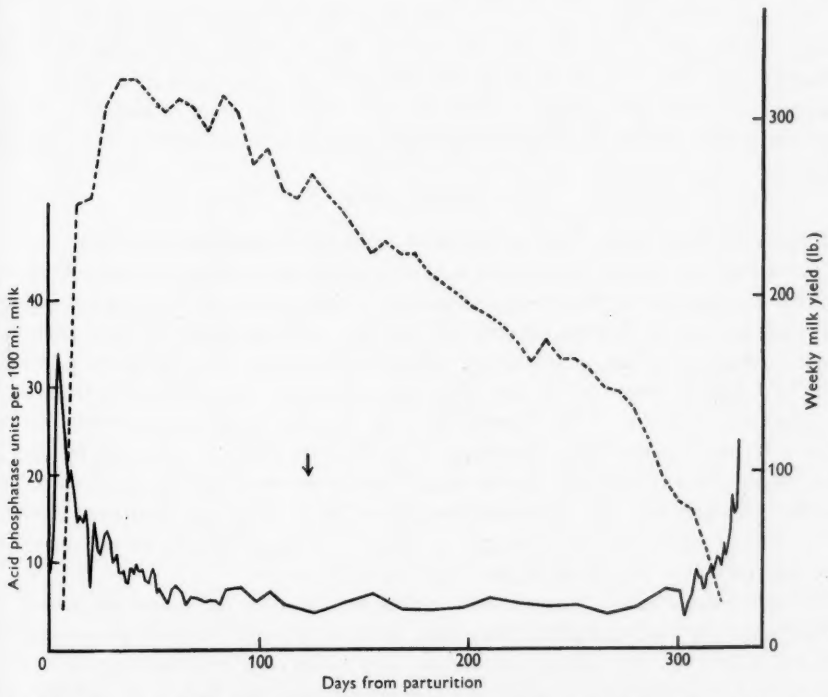

Fig. 4. Rosalie 14. The relation between the acid phosphatase concentration (full line), the weekly milk yield (dotted line) and the stage of lactation. Conception date indicated by arrow.

DISCUSSION

From Table 1 it will be seen that 88% of the individual milks gave phosphatase titres of between 2 and 8 units per 100 ml. milk, while 80% of the bulk milk gave phosphatase titres between 4 and 8 units per 100 ml. milk.

In milks from cows early in lactation, concentrations greater than 10 units % were found on many occasions in the four cows which were investigated throughout lactation. Prior to 60 days after parturition, such concentrations in single cow samples are common.

From Figs. 1–4 it is clear that the enzyme concentration follows a definite pattern throughout lactation. From a low initial value there was an increase up to the 5th–6th day, after which a steady decline occurred until the 100th day. This level was maintained until about 30 days before drying off, when an increase in the concentration began to take place.

Attempts were made to correlate these changes with changes in the concentration of other milk constituents, namely, alkaline phosphatase, solids-not-fat and albumin and globulin nitrogen, and these are discussed below.

Alkaline phosphatase

The pattern of concentration of acid phosphatase is very different from that found for alkaline phosphatase by Folley & Kay (3). These workers found that from an initial high titre there is a sharp drop to a minimum between 10 and 25 days after parturition, after which a steady increase is maintained to the end of lactation. Total alkaline phosphatase production increases to a peak at about 180 days after parturition and gradually decreases, while total acid phosphatase production decreases from the start of lactation. There is therefore no close relationship between the concentration of these two enzymes throughout lactation.

Solids-not-fat

Of the four cows individually investigated, frequent estimations of solids-not-fat were not carried out beyond the 60th day of lactation, except at monthly intervals. From these results it was possible to obtain correlation coefficients between the enzyme concentration and solids-not-fat % between the 4th and 7th day until the 60th day after parturition. These coefficients (r) are given below. Clara 11: from the 4th day after parturition, $r = 0{\cdot}4031$; Lily 7: from the 7th day after parturition, $r = 0{\cdot}388$; Flora 70: from the 6th day after parturition, $r = 0{\cdot}344$; Rosalie 14: from the 5th day after parturition, $r = 0{\cdot}440$.

Clara 11 and Rosalie 14 gave correlation coefficients between solids-not-fat and acid phosphatase concentration which were significant at less than the 5% level. Lily 7 gave a coefficient which was just significant, and Flora 70 did not give a significant coefficient, but pooling all these coefficients by the 'z' transformation (Fisher (5)) a coefficient of 0·39 is obtained which is significant at less than the 0·1% level.

Bailey (6) has correlated the increase in solids-not-fat after the 3rd or 4th month after parturition with the conception date. The increase in acid phosphatase concentration does not occur until late in lactation, and the correlation coefficient between solids-not-fat and enzyme concentration between the conception date (see Figs. 1–4) and the end of lactation was $r = 0{\cdot}362$. This figure indicates chances of 15 to 1 in favour of a relationship.

Albumin plus globulin nitrogen

Azarme(7) has very comprehensively surveyed the total protein nitrogen, casein nitrogen and albumin plus globulin nitrogen throughout lactation of the cow. Of the three, the last is perhaps the most similar to the curves for acid phosphatase. In the first 5–6 days of lactation, there appears to be no parallel between the acid phosphatase concentration and the albumin plus globulin fraction, as this fraction is decreasing from parturition (Richmond, Elsdon & Walker(8)); from then on both levels maintain a tolerably parallel course. This argument could be applied to other milk proteins, but the mid-lactation level of albumin plus globulin is more steady than the other fractions. However, the albumin plus globulin fraction reaches a minimum 30 days after parturition, while acid phosphatase does not do so until 60–100 days.

There appears to be a strong inverse relationship between the stage of lactation and the total yield of acid phosphatase at the morning milking (see Table 2).

SUMMARY

1. Nearly 90% of the cows (between 50 days after parturition and prior to 30 days before drying off) investigated gave milk with titres between 2 and 8 Gutman acid phosphatase units per 100 ml. milk.

2. Milk acid phosphatase concentration follows a reasonably uniform pattern from cow to cow during the progress of lactation. Very high values are found in early lactation.

3. No conclusive correlation between the acid phosphatase concentration and that of other milk constituents studied was observed throughout a *whole* lactation. A correlation did exist between the enzyme concentration and solids-not-fat after the first few days of lactation to about the 60th day. Throughout a lactation there is a fairly close relationship between the changes in enzyme concentration and albumin plus globulin nitrogen.

4. There is an inverse relationship between advancing stage of lactation and the total quantity of phosphatase in the morning yield of milk.

I wish to thank Mr A. Wagstaff for estimating solids-not-fat.

REFERENCES

(1) MULLEN, J. E. C. (1950). *J. Dairy Res.* **17**, 288.
(2) FOLLEY, S. J. & KAY, H. D. (1936). *Ergebn. Enzymforsch.* **5**, 159.
(3) FOLLEY, S. J. & KAY, H. D. (1936). *Enzymologia*, **1**, 48.
(4) GUTMAN, E. B. & GUTMAN, A. B. (1940). *J. biol. Chem.* **136**, 201.
(5) FISHER, R. A. (1938). *Statistical Methods for Research Workers*, 7th ed. Edinburgh: Oliver and Boyd.
(6) BAILEY, G. L. (1948). Private communication.
(7) AZARME, E. (1938). *J. Dairy Res.* **9**, 121.
(8) RICHMOND, H. D., ELSDON, G. D. & WALKER, G. H. (1942). *Richmond's Dairy Chemistry*, 4th ed. London: Charles Griffin and Co. Ltd.

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III. THE DISTRIBUTION OF ACID PHOSPHATASE IN THE MILK FROM INDIVIDUAL QUARTERS OF THE UDDER OF THE COW AND ITS RELATIONSHIP TO SUBCLINICAL MASTITIS

Parts I and II have demonstrated some of the characteristic properties of the acid phosphatase of cows' milk. It has been shown by many workers—reference may be made to McDowall (3)—that the concentration of various constituents of cows' milk may be greatly altered when that milk comes from an infected quarter. Whilst milk from the different quarters of an apparently normal, healthy udder is not necessarily of exactly the same composition, the variations from one quarter to another are usually small. It was decided to investigate the extent of these variations in acid phosphatase in the quarters of normal and infected udders.

EXPERIMENTAL

The cows in the herd of the Institute farm were studied. Samples were taken from each quarter about half a minute after the start of milking. Acid phosphatase was estimated by the procedure employed by Mullen (1). The unit of enzyme activity has been defined by Gutman & Gutman (4).

During the first part of this investigation high values were obtained in those quarters of the udder which were infected. With this information at hand, experimental procedure was altered and samples of milk were taken aseptically from all cows prior to ordinary milking, and their acid phosphatase titres determined.

Bacteriological tests were carried out on each sample by the following technique: 0·04 ml. of milk was streaked on the surface of nutrient blood and aesculin agar, and incubated for 40 hr. at 37° C., after which identification and counts were made.

These experiments indicated that high acid phosphatase values were associated with *Streptococcus agalactiae* infections. Consequently *Str. agalactiae* was grown from the milk of a cow (Niobe) with persistent infection of her left-hind quarter in dextrose Lemco broth for 24 hr. The bacterial suspension was centrifuged gently and the supernatant clear liquid pipetted off, leaving *c.* 8 ml. of broth, in which the bacteria were resuspended. This suspension was then examined for phosphatase activity at pH 4·1 by the normal procedure.

The samples of milk from the four quarters of Niobe were examined for acid phosphatase not more than 15 min. after sampling. Duplicates of the same samples were pasteurized at 62·8° C. for 30 min., and samples of the raw and pasteurized milks were then maintained at 3 and at 37° C. for 24 hr. Acid phosphatase estimations were carried out on the milks after pasteurization and after the 24 hr. incubation of both series at the two temperature levels.

RESULTS

Twenty-six cows which had no infection of the udder during their lactation were examined; two of these, Winnie 11 and Flora 74, had had an infection in their left-hind quarters in their previous lactation. The milk acid phosphatase titres of these cows are given in Table 1.

Eight cows with infection in one or more quarters were also investigated and the titres are given in Table 2. The titres of eight cows that were not infected at the time of sampling but had a history of infection during the lactation are given in Table 3.

The results of the examination of the milk from the four quarters of Niobe are shown in Table 4.

Table 1. *The concentration of milk acid phosphatase in units* 100 *ml. milk in the four quarters of the udder*

Cow	Quarters R.F.	L.F.	R.H.	L.H.	Bulk	Difference between highest and lowest quarter
Campion 12	6·5	6·6	7·4	7·4	7·1	0·9
Campion 14	2·8	2·7	2·9	2·8	3·0	0·2
Clara 12	6·0	6·7	6·4	6·0	6·2	0·7
Doris 6	4·3	5·2	7·0	6·0	7·5	2·7
Dorritt 6	8·0	7·4	7·8	7·2	6·7	0·8
Dorritt 7	5·1	4·8	4·8	5·5	5·2	0·7
Dorritt 8	6·8	7·4	6·9	7·1	7·4	0·6
Flora 74	3·5	4·8	10·3	5·5	—	6·8
Flora 76	1·9	3·5	2·9	3·7	3·8	1·8
Hecuba	2·9	3·3	Blind	2·2	3·4	1·1
Lottie 16	4·6	3·5	4·1	4·0	3·9	1·1
Peach 20	4·7	6·0	Blind	6·2	6·2	0·5
Peach 24	5·2	5·0	5·2	4·9	5·2	0·3
Peach 25	4·9	5·1	4·8	4·9	4·9	0·3
Pearl 7	9·0	10·7	Blind	13·7	11·6	4·7
Pearl 11	2·3	2·6	2·8	2·5	2·8	0·5
Pippin 8	3·3	4·4	3·2	3·0	3·5	1·4
Rosalie 23	4·1	4·3	4·4	4·6	4·5	0·5
Rosetta 6	3·4	3·6	4·0	4·5	3·9	1·1
Rosey 10	2·4	3·0	2·4	2·4	2·5	0·6
Rosey 11	7·3	4·7	4·3	3·4	5·1	3·9
Winnie 11	3·9	3·2	3·7	3·6	3·9	0·7
Winnie 13	10·0	10·1	13·0	16·1	10·0	6·0
Winsome 19	2·7	2·8	3·1	3·0	2·8	0·4
Winsome 20	8·8	8·0	8·3	7·2	8·8	1·6
Winsome 21	3·2	2·5	2·5	2·9	2·7	0·7

Table 2. *The acid phosphatase titres of milk from infected and uninfected quarters of the udder of the cow, together with the results of bacteriological examinations*

Cow	Quarters R.F.	L.F.	R.H.	L.H.	Bulk
Campion 11	9·4	32·7 *Str. agalactiae*	7·8	10·0	13·1
Campion 14	55·0 5000 *Str. agalactiae*	3·1	3·0	2·2	—
Flora 43	9·0	12·2 10,000 Gp. III *Str.*	17·3 Gp. III *Str.*	9·5 3750 micrococci	9·9
Flora 45 (i)	7·2	9·9 Gp. I *Str.*	7·5 Haem. *Staph.*	14·0 Gp. I *Str.*	9·3
Flora 45 (ii)	8·2	16·9 25,000 *Str. agalactiae*	11·5 5000 Gp. III *Str.*	38·6 20,000 *Str. agalactiae*	—
Hera	2·8	3·1	2·8	7·2 750 *Str. agalactiae*	—
Lily 7	5·4	6·2	5·5	17·8 2500 *Str. agalactiae*	—
Niobe	3·4	4·1	4·1	8·5 7500 *Str. agalactiae* 500 Haem. *Staph.*	—
Truth	9·9 Gp. III *Str.*	3·7	13·8 Gp. III *Str.*	4·6	7·4

Table 3. *The acid phosphatase titres of milk from quarters with a history of infection, together with details of the infection*

Cow	Quarters R.F.	L.F.	R.H.	L.H.	Bulk	Difference between highest and lowest quarter
Flora 62	7·6	12·0	10·2	11·0	7·3	4·4
	History of Gp. II *Str.* in R.H.Q.					
Flora 69	2·0	2·2	4·8	2·6	2·6	2·8
	History of micrococci in R.H.Q.					
Flora 74	2·9	2·8	2·9	2·5	3·1	0·4
	History of Gp. I *Str.* in L.H.Q.					
Hebe	2·5	2·7	2·8	2·6	4·2	0·3
	History of Gp. I *Str.* in R.F.Q. and Gp. III *Str.* in L.H.Q.					
Hera	3·9	4·3	4·1	4·3	4·6	0·4
	History of Gp. II *Str.* in L.H.Q.					
Peach 23	6·4	7·3	6·6	6·8	7·4	0·9
	History of Haem. *Staph.* in L.F.Q.					
Pippin 3	2·8	7·7	3·6	5·9	4·7	4·9
	History of Gp. III *Str.* in R.F.Q., L.H.Q.					
Winsome 21	3·5	3·5	11·7	4·4	—	8·2
	History of Haem. *Staph.* in R.H.Q.					

Table 4. *The acid phosphatase titres of milk from the infected and uninfected quarters of Niobe, before and after pasteurization and after keeping the milks at* 3 *and* 37° C. *for* 24 *hr. Bacteriological results are also given*

	Quarters R.F.	L.F.	R.H.	L.H. (infected)
	Raw milk			
Initial	7·2	6·0	7·8	8·3
After 24 hr.:				
(i) at 3° C.	6·4	5·8	7·4	8·1
(ii) at 37° C.	6·2	5·7	6·1	10·2
	Pasteurized milk			
Initial	4·9	3·6	5·7	6·0
After 24 hr.:				
(i) at 3° C.	3·9	6·1	5·0	5·0
(ii) at 37° C.	4·7	3·4	4·7	4·0
	Raw milk, bacteriological findings			
Corynebacterium/ml.	4000	800	4000	Few
Strep. agalact./ml.	Nil	Nil	Nil	20,000

DISCUSSION

From Table 1 it appears that twenty-one of the twenty-six cows investigated gave a maximum difference between the highest and lowest phosphatase titre of less than 2 units per 100 ml. milk, while five cows gave a maximum difference of over 2 units %.

It will be observed from Table 2 that *all* the cows infected gave increased acid phosphatase titres in the milks from the infected quarter. The most common infecting organism was *Str. agalactiae*, and the highest titres were associated with the presence of this organism.

Of the eight cows with history of infection (Table 3) only three gave no increase in acid phosphatase titres. Of the remainder, three gave an increase in the previously infected quarter and two gave rather puzzling results.

In connexion with the association of *Str. agalactiae* with high acid phosphatase titres as shown in Table 2, it is interesting to note that the milk from the left-hind quarter of Niobe (Table 4) has an increased (23%) phosphatase titre after 24 hr. at 37° C. The pasteurized sample from the same quarter did not show this increase (Mullen (1, 2) has shown that pasteurization causes only partial destruction of acid phosphatase) and no increase was observed in the milk from the other quarters. In view of the bacteriological tests carried out on these samples, and that a milk-free suspension of *Str. agalactiae* was capable of hydrolysing phenylphosphate at pH 4·1, it seems highly probable that the presence of the living organism is responsible for the higher titre.

Since over 70% of the uninfected cows gave less than 1 unit % difference while nearly 90% gave less than 4 units % difference, a milk acid phosphatase titre from a quarter of the udder of more than 4 units higher than that of other quarters, points to the probability of an infection of that quarter.

SUMMARY

1. The acid phosphatase content of milk is reasonably constant from quarter to quarter in normal uninfected cows.

2. The acid phosphatase content of milk from infected quarters is enhanced in comparison with the uninfected quarters. The differences found were from 4·1 to over 50 enzyme units per 100 ml. milk.

3. It has been shown that a *Streptococcus agalactiae* suspension is capable of hydrolysing phenylphosphate at pH 4·1, and it is suggested that this organism is largely responsible for the enhanced enzyme content of the milk from the infected udder.

I am grateful to Mr F. K. Neave for the bacteriological work undertaken.

REFERENCES

(1) MULLEN, J. E. C. (1950). *J. Dairy Res.* **17**, 288.
(2) MULLEN, J. E. C. (1950). *J. Dairy Res.* **17**, 295.
(3) MCDOWALL, F. H. (1945). *N.Z. J. Sci. Tech.* **27** (sec. A), 258.
(4) GUTMAN, E. B. & GUTMAN, A. B. (1940). *J. biol. Chem.* **136**, 201.

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428. THE ACID PHOSPHATASE OF MAMMARY TISSUE OF THE COW AND THE RAT

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(With 1 Figure)

Greenstein (1) has shown that the mammary tissue of the mouse contains both acid and alkaline phosphatase. Using phenylphosphate as a substrate, the activity in the acid range was measured at pH 4·6. No optimum pH had been determined.

Dempsey, Bunting & Wislocki (2), using a histological method, demonstrated the presence of an acid phosphatase in the mammary tissue of the rat and the woman. The pH they employed during hydrolysis was 4·7.

Caputto & Marsal (3), using β-glycerophosphate as a substrate, have shown that mammary tissue of the cow is rich in *alkaline* phosphatase. This enzyme is also present in the mammary tissue of the guinea-pig (Folley & Kay (4)) and of the rat (Folley & Greenbaum (5)).

The present paper shows that mammary tissue of the cow contains an acid phosphatase and confirms the presence of an acid phosphatase in mammary tissue of the rat. An examination of the effect of adrenalectomy and replacement therapy on the acid phosphatase of rat mammary tissue is also reported.

EXPERIMENTAL

Materials

Fresh mammary tissue from cows was obtained from a local slaughterhouse. A weighed amount of the tissue (about 5 g.) was dispersed in 19 times its weight of normal saline (0·85 g. NaCl/100 ml. dist. water) by treatment in a tissue homogenizer as described by Folley & Watson (6). The resulting extract was filtered through glass-wool and the filtrate used for investigations.

Rat mammary tissue was obtained by careful dissection of the abdominal glands. The tissue underwent the same treatment as described above.

Rat mammary tissue was also available from individual rats which had undergone adrenalectomy and replacement therapy with cortical steroids. At parturition, sixty rats were divided into five groups; the number of young was standardized by the method described by Folley & Cowie (7), all litters being reduced to eight at birth, four pups of each sex being preserved whenever possible.

The groups were subjected to the following treatment: (*a*) controls (subjected to a sham operation); (*b*) adrenalectomy carried out on the 4th day of lactation; (*c*) adrenalectomy +0·56 mg. 11-dehydrocorticosterone acetate (DHCA) per day; (*d*) adrenalectomy +0·56 mg. deoxycorticosterone acetate (DOCA); (*e*) adrenalectomy+3·0 mg. DOCA. Groups (*a*) and (*b*) were injected daily with sesame oil. The cortical steroids injected in groups (*c*), (*d*) and (*e*) were dissolved in sesame oil.

Each group was divided into two halves, one of which was fed a stock diet (0·33% Na; 0·88% K) and the other on a high sodium diet (0·67% Na; 0·88% K).

All mothers were killed on the 17th day of lactation by dislocation of cervical vertebrae, and the removal of the mammary glands and kidneys was carried out. Mammary gland homogenates were prepared by the method described above. The kidneys were also removed and dispersed in 39 times their weight of normal saline.

Methods

The method of Gutman & Gutman (8) was adopted with appropriate adjustment of the pH, after the optimum had been determined. Their definition of a phosphatase unit was also used. pH measurements were made by a direct-reading Cambridge pH meter using a glass electrode.

Kidney acid phosphatase was estimated at pH 5·5 by a similar procedure, this pH having been found by experiment to be about the optimum.

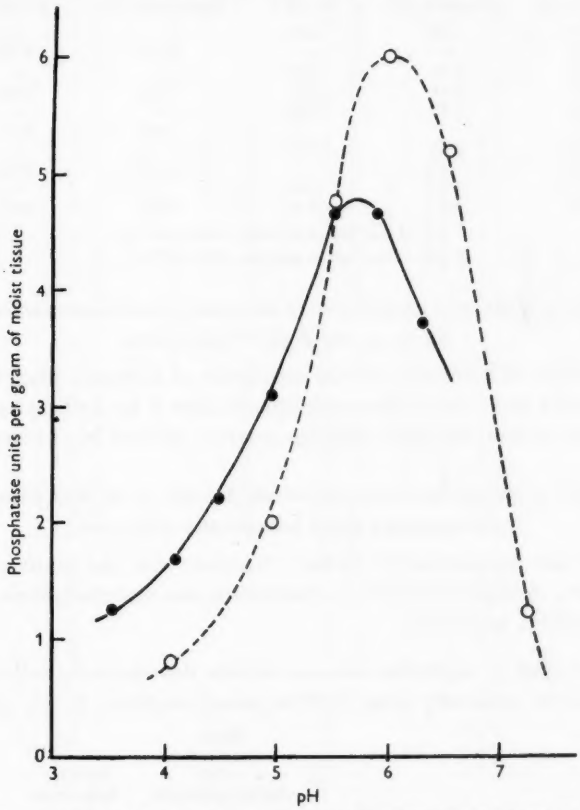

Fig. 1. Activity-pH curves for acid phosphatase of cows' mammary tissue (full line) and rats' mammary tissue (broken line). Hydrolysis at 37° C. in the presence of 0·005-M substrate. Reaction time 1 hr.

RESULTS

The effect of pH *on the activity of the acid phosphatases*

In Fig. 1, the peak of activity of the enzyme in mammary tissue homogenates from the cow lies between pH 5·5 and 5·8, while for the rat an optimum at about pH 6·0 is observed.

The rate of hydrolysis by the acid phosphatases

Table 1 gives the values of the reaction constant as calculated from the equation

$$k = \frac{2{\cdot}303}{t} \log \frac{a}{a-x},$$

where t is the time of hydrolysis, a is the initial concentration of the substrate, and x the amount hydrolysed in the time t.

Table 1. *Hydrolysis of* 0·005 M-*phenylphosphate by mammary tissue homogenate acid phosphatase at* 37° C.

	Cow*		Rat†	
Time in min.	Amount hydrolysed (%)	$k \times 10^{-4}$	Amount hydrolysed (%)	$k \times 10^{-4}$
10	0·48	4·88	—	—
15	—	—	0·56	3·79
20	1·04	5·22	—	—
30	1·48	4·81	0·98	3·29
40	1·72	4·36	—	—
45	—	—	1·26	2·81
50	2·35	4·32	—	—
60	—	—	1·63	2·74
76	2·9	3·88	—	—
120	4·2	3·50	2·80	2·37

* pH of incubation mixture, 5·68 at 20° C.

† pH of incubation mixture, 6·04 at 20° C.

The action of the acid phosphatase in mammary gland homogenates of the cow on α- and β-glycerophosphates

Using the method of Sumner (9) for the estimation of inorganic phosphate, hydrolysis of 0·005 M solutions of α- and β-glycerophosphate after 5 hr. hydrolysis was measured. The β-isomer was hydrolysed faster than the α-salt, a ratio of 2·3:1 being observed.

The effect of magnesium ions and sodium fluoride on the acid phosphatase in mammary gland homogenates of the cow

These results are summarized in Table 2 together with the limits of concentration causing the effect. A slight inhibition by magnesium was observed, while sodium fluoride caused just over 40% inhibition.

Table 2. *The effect of magnesium ions and sodium fluoride on the activity of the acid phosphatase in cows' mammary tissue* (0·005 M-*phenylphosphate;* 37° C.; pH 5·7 *at* 20° C.)

	Molar concentration $\times 10^3$ during hydrolysis	Relative hydrolysis
Magnesium chloride	0	1·00
	0·139	0·97
	0·278	0·95
	1·39	0·91
Sodium fluoride	0	1·00
	2·27	0·68
	4·54	0·57

The enzyme was also inhibited by raw milk to the extent of about 50%. Boiled milk did not have this effect. The enzyme in the homogenate was also affected by light, about

Table 3. *The effect of adrenalectomy and replacement therapy on the mean acid phosphatase levels (measured in Gutman & Gutman (8) units/g. moist tissue) of mammary tissue of lactating rats*

	Treatment									
	Control		Adrenalectomy		Adrenalectomy +0·56 mg. DOCA		Adrenalectomy +3·0 mg. DOCA		Adrenalectomy +0·56 mg. DHCA	
Fisher's '*P*' for results with	0·33% Na 12·38±1·07	0·67% Na 14·05±0·59	0·33% Na 10·31±0·94	0·67% Na 11·7±1·25	0·33% Na 10·5±0·64	0·67% Na 11·83±0·85	0·33% Na 11·2±0·89	0·67% Na 11·43±0·77	0·33% Na 9·32±1·31	0·67% Na 10·38±1·00
(1) Control 0·33% Na	—	—	0·1646	—	0·1646	—	0·4406	—	0·102	—
(2) Control 0·67% Na	—	—	—	0·1172	—	0·062	—	**0·0224**	—	**0·0094**
(3) Adrenalectomy 0·33% Na	—	—	—	—	0·8454	—	0·4998	—	0·5618	—
(4) Adrenalectomy 0·67% Na	—	—	—	—	—	0·9222	—	0·8452	—	**0·4406**

Significant values of '*P*' are given in heavy type.

Table 4. *The effect of adrenalectomy and replacement therapy on the mean acid phosphatase level (measured in Gutman & Gutman (8) units/g. moist tissue) of the kidneys of lactating rats*

	Treatment									
	Control		Adrenalectomy		Adrenalectomy +0·56 mg. DOCA		Adrenalectomy +3·0 mg. DOCA		Adrenalectomy +0·56 mg. DHCA	
Fisher's '*P*' for results with	0·33% Na 28·03±0·92	0·67% Na 28·5±0·57	0·33% Na 25·78±0·9	0·67% Na 24·7±1·18	0·33% Na 24·67±0·75	0·67% Na 24·22±0·5	0·33% Na 26·81±1·35	0·67% Na 23·97±1·28	0·33% Na 25·75±0·71	0·67% Na 26·28±0·71
(1) Control 0·33% Na	—	—	0·102	—	**0·0188**	—	0·4984	—	0·0734	—
(2) Control 0·67% Na	—	—	—	**0·0144**	—	**0·0002**	—	**0·0158**	—	0·0866
(3) Adrenalectomy 0·33% Na	—	—	—	—	0·3892	—	0·5606	—	1·000	—
(4) Adrenalectomy 0·67% Na	—	—	—	—	—	0·6968	—	0·6968	—	0·2578

Significant values of '*P*' are given in heavy type.

50% being destroyed after 15 min. exposure to a quartz mercury vapour lamp (220 V., 800 W.) at a distance of 15 cm.

The effect of adrenalectomy and of cortical steroids after adrenalectomy on the acid phosphatase of mammary tissue during lactation

Table 3 gives the mean levels of mammary tissue acid phosphatase of various groups undergoing different treatments together with the standard deviations. Fisher's 'P' has been calculated and is also given. Table 4 gives mean levels of kidney acid phosphatase. In this experiment the kidney was used as a control organ.

DISCUSSION

The mammary tissue homogenates of the cow and rat contain an acid phosphatase with pH optima between 5·5 and 6·0 and at 6·0 respectively. Hydrolysis of the glycerophosphates by homogenates of mammary tissue of the cow show that the enzyme hydrolyses the β-salt at a faster rate than the α-salt. From the classification of the phosphatases by Folley & Kay (10), this enzyme would appear to belong to group AII. This is further substantiated when the non-activating effect of magnesium is taken into account. The acid phosphatase of rat mammary tissue appears also to be a member of this group of the phosphatases.

A zero order of reaction for cow mammary tissue acid phosphatase appears evident up to about 30 min. hydrolysis, while a first-order reaction appears evident for rat mammary tissue acid phosphatase. The decreases in the rates of hydrolysis may be due to enzyme destruction brought about by the effects of temperature or pH.

The effect of sodium fluoride on the enzyme is not so noticeable as the effect of this inhibitor on milk acid phosphatase (Mullen (11)).

The factor in raw milk responsible for inhibition of mammary tissue acid phosphatase is obviously destroyed by heat, and in view of this inhibition observed in the presence of the milk, and together with the other dissimilarities with milk acid phosphatase (see Mullen (11)), it seems likely that the two enzymes are not identical.

From Table 3 it is seen that no marked differences for mammary tissue phosphatase in the various treatments were observed. A slight decrease, though not statistically significant (taking $P \leqslant 0{\cdot}05$ as the generally accepted level of significance), occurred as a result of adrenalectomy. The decreases observed on the high sodium diets appear more significant than those on the low sodium diets. Only two significant decreases were observed, namely, with adrenalectomy + 0·56 mg. DHCA and 3·0 mg. DOCA.

Kidney acid phosphatase exhibited a decrease, and again the decreases of those groups on a high sodium diet were more significant. These are shown in Table 4.

Folley & Greenbaum (12) have shown that adrenalectomy caused a reduction in liver and mammary gland arginase and kidney alkaline phosphatase, but not mammary gland alkaline phosphatase. They have also shown that the administration of cortical steroids has caused partial restoration of liver arginase, while mammary gland arginase was not restored. Kidney alkaline phosphatase was almost totally restored.

The acid phosphatase content of rat mammary tissue, measured on a basis of units per g. moist tissue, is about 50% that of the level in the kidneys.

SUMMARY

1. Mammary tissue of the cow and the rat contains acid phosphatase. The respective pH optima are 5·5–5·8 and 6·0.

2. The enzyme in cow mammary tissue is probably one of type AII in the Folley & Kay (10) classification.

3. The acid phosphatase of cow mammary tissue is inhibited by a factor in raw cows' milk. This factor is destroyed by heat.

4. On the basis of rate of hydrolysis of phenylphosphate at the optimum pH there is about 5 times more alkaline phosphatase in the mammary tissue of the cow than acid phosphatase.

5. The effect of adrenalectomy and replacement therapy through the administration of cortical steroids has no marked effect on the acid phosphatase of rat mammary tissue.

I wish to thank Dr S. J. Folley for access to the physiological material used in the experiments on rat mammary gland acid phosphatase.

REFERENCES

(1) GREENSTEIN, J. P. (1942). *J. nat. Canc. Inst.* **2**, 511.
(2) DEMPSEY, E. W., BUNTING, H. & WISLOCKI, G. B. (1947). *Amer. J. Anat.* **81**, 309.
(3) CAPUTTO, R. & MARSAL, A. (1941). *Rev. Soc. argent. Biol.* **17**, 139.
(4) FOLLEY, S. J. & KAY, H. D. (1935). *Biochem. J.* **29**, 1837.
(5) FOLLEY, S. J. & GREENBAUM, A. L. (1947). *Biochem. J.* **41**, 261.
(6) FOLLEY, S. J. & WATSON, S. C. (1948). *Biochem. J.* **42**, 204.
(7) FOLLEY, S. J. & COWIE, A. T. (1944). *Yale J. biol. Med.* **17**, 67.
(8) GUTMAN, E. B. & GUTMAN, A. B. (1940). *J. biol. Chem.* **136**, 201.
(9) SUMNER, J. B. (1944). *Science*, **100**, 413.
(10) FOLLEY, S. J. & KAY, H. D. (1936). *Ergebn. Enzymforsch.* **5**, 159.
(11) MULLEN, J. E. C. (1950). *J. Dairy Res.* **17**, 288.
(12) FOLLEY, S. J. & GREENBAUM, A. L. (1946). *Biochem. J.* **40**, 46.

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637.35

429. STUDIES ON EGYPTIAN DOMIATI CHEESE

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(With 1 Plate and 10 Text-figures)

I. GENERAL METHODS OF MANUFACTURE

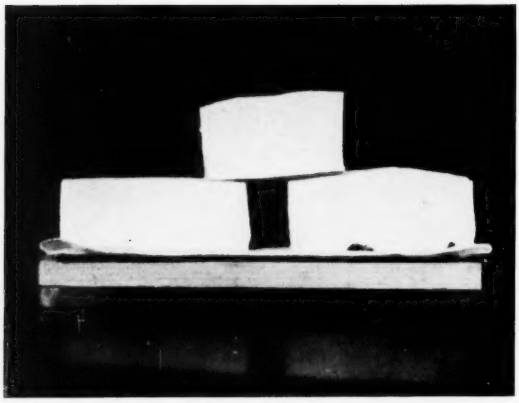
Although the 'pickled' varieties of cheese (which include Domiati) are of great importance in warm climates, they are not included in the usual cheese classifications and have received little mention in the scientific literature. They are well suited to both the climatic and economic conditions of the Balkans and Near East, where they are manufactured extensively.

The pickled group comprises soft uncoloured cheese, ripened without mould, which may be consumed fresh but more usually after maturation in salt solution in sealed containers.

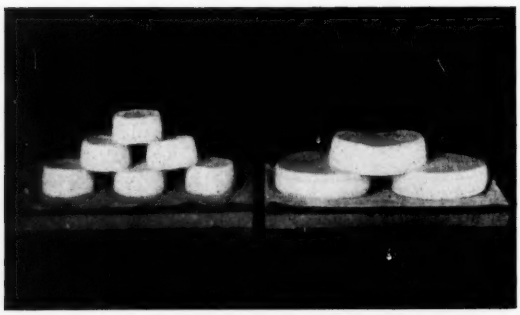
The salted solution or 'pickle' (usually salted whey) improves the flavour of the cheese and acts also as a preservative. This pickling practice constitutes the main difference between the group and other typical soft cheese of colder zones. Under warm atmospheric conditions, when the keeping quality of milk is short and cheese soon deteriorates before it ripens, salting becomes one of the inevitable practices necessary for its preservation. The most important varieties of soft pickled cheese are *Feta* and *Teleme* (Greece), *Salamora* (Bulgaria and Yugoslavia), *Brandza de Braila* (Roumania), *Domiati* and *Kareish* (Egypt). Table 1 illustrates the main differences between *Feta*, *Teleme*, *Domiati* and *Kareish* cheese. *Salamora* and *Brandza de Braila* are very similar to *Teleme*.

Table 1. *Summary of methods of manufacture of 'pickled' varieties of soft cheese*

	Varieties			
	Teleme	Feta	Domiati	Kareish
Kind of milk used:				
Full cream cows' milk	+ or −	+ or −	+ or −	−
Full cream buffaloes' milk	−	−	+ or −	−
Full cream ewes' milk	+	+	−	−
Full cream goats' milk	+ or −	+ or −	−	−
Full cream mixed milk	+ or −	+ or −	+ or −	−
Separated milk added	+ or −	+ or −	+ or −	−
Skimmed milk only	−	−	−	+
Coagulation:				
Rennet	+	+	+	−
Acid	−	−	−	+
Salting:				
Milk salted prior to rennetting	−	−	+	−
Curd salted during drainage	−	+	−	−
Cheese salted with dry salt after drainage	+ or −	+ or −	−	+
Cheese salted in brine tank	+	−	−	−
Salted solution added to preserving container	+	+	+	+
Storage:				
In tins	+	−	+	−
In wooden barrels	−	+	−	−
In earthenware vessels	−	−	−	+



(*a*) Cheese from square wooden forms.



(*b*) Cheese from cylindrical moulds.

Domiati cheese

Domiati cheese, one of the most popular cheeses of Egypt, is known to some extent in some other Arabic-speaking countries but not elsewhere. In spite of the special technique used in its manufacture, which renders it particularly suited to warm climatic conditions and low-quality milk supplies, no reference to this variety has been found in European or American literature (1).

It differs chiefly from other pickled varieties (Table 1) in being salted at the very first step in its manufacture, when salt is added directly to the milk, whereas in the other varieties salting takes place at a later stage during or after the drainage process.

Shape

The shape of finished Domiati cheese varies widely with the curd forms used for whey drainage. The cheese may be rectangular (Pl. 1*a*) or cyclindrical (Pl. 1*b*). The former shape is more common in commercial practice, as it is frequently packed into rectangular petrol tins, when the cheese is put in layers, nine pieces per layer, each piece measuring 8 cm. in length and breadth by 3·5 cm. in thickness. When cyclindrical in shape, the cheese may be produced in different dimensions, e.g. 12 cm. in diameter by 3·5 cm. in thickness or 6 cm. in diameter by 4 cm. in thickness.

Flavour

Domiati has a distinctive flavour, mild and rather salty when fresh and later a little clean desirable acidity when ripened. When old (over 1 year) Roquefort and old Cheddar-like flavours develop, accompanied by a change of colour from white to light brown. After still further ripening, the flavour becomes more pronounced and is accompanied by strong odours and further darkening of colour.

Body and texture

Fresh Domiati cheese has a soft body (a little softer than that of fresh Coulommier cheese). As it ripens in the 'pickle', the body usually becomes firmer up to the third month after which it 'mellows', mainly as a result of protein breakdown. Domiati cheese should possess a close texture with no holes; as ripening proceeds it becomes slightly flaky and is brittle rather than elastic when broken.

It should be added that, apart from age and ripening, the body, texture and flavour of the cheese are influenced by its chemical composition, the flora present, the composition of the pickling mixture, and the temperature during storage and ripening.

Methods of manufacture

Milk used

Domiati cheese is made from whole buffaloes' or cows' milk or from a mixture of varying proportions of whole and separated milk. Buffaloes' milk (constituting 70% of the total output in Egypt (2)) is preferred, since whole buffaloes' milk, with its average 7% fat content, produces a rich cheese.

Salting

A relatively high percentage of salt (5–15%) is added directly to the milk prior to rennetting. The addition of salt tends to counteract the high microbial contamination

which usually prevails during the production of milk and its manufacture into cheese, and which is accentuated by rapid bacterial growth at relatively high temperature. The exact salt addition varies, being increased with higher atmospheric temperature, lower quality milk and longer storage periods. The salted milk is strained through muslin cloth to free it from visible impurities.

Salting cheese milk prior to rennetting is not entirely unknown in other varieties. Thus, it is occasionally practised in the manufacture of other varieties of soft cheese, e.g. Pont l'Evêque, and has been referred to by Davis(3) in making single cream cheese and by Sammis in the manufacture of Cheddar cheese(4). In these instances, salting the milk before rennetting is an exceptional temporary measure, employed to counteract the adverse effects of high temperature and contamination of milk, whilst in Domiati cheese it is normal practice.

Heating

Salted milk may be heated before rennetting in different ways, e.g.

(1) Warming directly to 95–105° F.

(2) Heating to 150° F. for about 15 min. and then cooling to 95–105°. This practice effects material reduction of the total bacterial count, avoidance of gas holes in the cheese and improvement of body and texture of the finished produce.

(3) The salt is added to two-thirds of the milk. The remaining third is heated to 170° F. for 5–10 min. and then mixed with the other portion. A final temperature of 105–115° F. is thus achieved which is suitable for rennetting. This method also improves cheese texture and body and slightly reduces the bacterial number.

Coagulation

Temperature: 95–115° F. Period: 2–3 hr. Amount of rennet: 10–15 ml. of good-quality 'standard' rennet per 100 lb. of milk.

The wide range of rennetting temperature is due to the varied means employed for holding milk during the coagulation period. With a double-jacketed cheese vat the range is narrower (95–100° F.), whilst with a non-insulated metallic tank or wooden barrel the range is wider to compensate for the drop of temperature during the clotting period. The unusually high temperature of coagulation and the relatively large addition of rennet have doubtless arisen mainly to counteract the effects of a high percentage of added salt, which greatly delays coagulation and markedly softens the body of the curd.

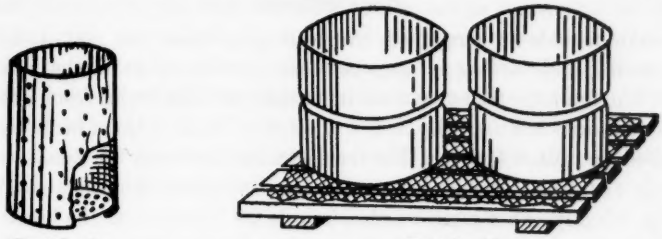
Ladling

When coagulated, the curd is ladled out into metallic moulds, wooden forms or frames.

Metallic moulds are cylindrical in shape and may be composed of one piece (Text-fig. 1*a*) 11 cm. in height and 8 cm. in diameter, or may be composed of 2 pieces similar to those used for Coulommier cheese, but rather smaller in size. When the two pieces fit together they measure 11 cm. in height, 12 cm. in diameter, the lower half of the mould being 7 cm. high (Text-fig. 1*b*). The metallic moulds are placed on suitable mats of mosquito net cloth, straw, sesaban wood or *Juncus* sp. stems. In turn the mats are placed over wooden boards. Before ladling out the curd into moulds, the top curd is skimmed off into a suitable container to be distributed evenly into the moulds when half full. The ladling process is similar to that used in Coulommier and other similar varieties of soft cheese.

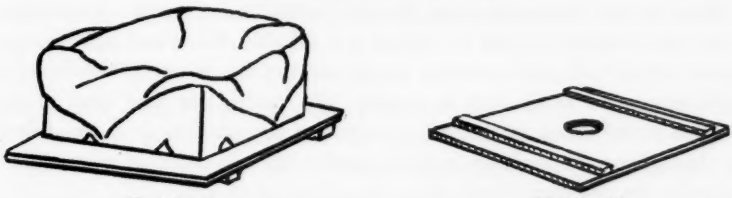
Wooden forms are rectangular in shape, devoid of base and top and with holes in their

sides. In height all types are approximately 20 cm., so that when curd drains sufficiently, the fresh cheese is 3·5–4·5 cm. in thickness, which favours a proper body and whey content. In length and breadth, however, the wooden forms differ, but a commonly used type is



Text-fig. 1*a* Text-fig. 1*b*

Text-fig. 1. *a*. Single cylindrical metallic moulds. *b*. Two double cylindrical metallic moulds placed on draining cloth and board.



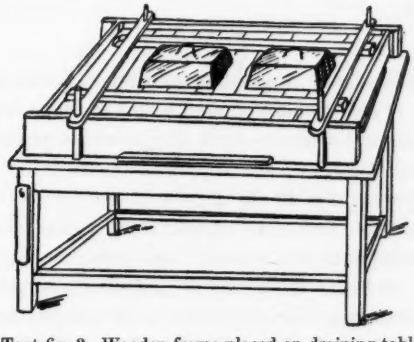
Text-fig. 2*a* Text-fig. 2*b*

Text-fig. 2*c* Text-fig. 2*d*

Text-fig. 2. *a*. Rectangular wooden form lined with drainage cloth and placed on a draining board. *b*. A square covering board. *c*. A cheese bundle placed between draining board and covering board. *d*. Pressing of cheese.

48–50 cm. in length and breadth, with a capacity of 100 lb. of curd. The wooden forms are placed on wooden boards, lined with mosquito net cloth (Text-fig. 2*a*), and the curd transferred into them by means of scoops.

In large cheese factories wooden frames of more ample dimensions are used. Such



Text-fig. 3. Wooden frame placed on draining table.

frames are composed of detachable wooden boards fixed over the draining table and are lined with mosquito net cloth. They hold up to 2000 lb. of curd (Text-fig. 3).

Whey drainage

When metallic moulds are employed, the drainage process, removal of the top half of the double mould, and turning of the cheese are performed as in the manufacture of Coulommier. The drainage times required in metallic moulds varies from 1 to 5 days.

When wooden forms are used the curd is allowed to drain in them for 3–4 hr., then the curd is bundled (in Stilton fashion). The forms are removed and the bundle covered with square boards (Text-fig. 2*b*) having central holes to serve as outlets for bundle ends (Text-fig. 2*c*). Finally, other plain wooden boards are placed over the bored boards and loaded with suitable weights to help drainage (Text-fig. 2*d*). The exact weight varies and is increased with delayed drainage (e.g. for 100 lb. of curd the weight may vary from 25 to 80 lb.). Time taken for drainage in wooden forms varies from 12 to 48 hr.

When large wooden frames are used, the curd is left to drain without external pressure for 5–10 hr., after which it is cut by means of a suitable shovel and lightly stirred. It is then covered with a cloth, and a wooden board carrying suitable weights to speed drainage. The weight employed varies, but is usually 50% more than that used in the bundle method, since in the frame method the presence of the side-boards completely enclosing the curd throughout the drainage process hinders the expulsion of whey. The drainage time in wooden frames varies from 12 hr. to as long as 48 hr.

The rate of drainage is variable and is governed by the usual factors, viz. increased by low fat or salt content, high acidity, increased rennet, firmness of curd, high temperature, openness of texture of cloth, pressure applied, number of drainage holes in boards and frames.

Preparation of fresh cheese for marketing

When the cheese is firm enough to be handled easily without damage to its shape, the metallic moulds are removed and the pieces of cheese are wrapped in water- and grease-proof paper. They may be packed in cartons for sale as fresh cheese.

In the case of wooden forms, weights and pressing boards are taken off, and the cheese turned and cut into 8 cm. square pieces.

When wooden frames are used, the weights, side-boards, pressing boards and cover are removed. The cloth covering the cheese is opened and the cheese cut as above. Pieces of cheese produced by the last-mentioned methods may also be wrapped for sale singly or in groups weighing 2–5 lb.

Pickling

Preservation of the green cheese in pickling solution or brine (in a similar way to that used in the manufacture of soft Balkan cheese) seems to be an invariable practice, owing to the relatively high moisture content—over 45%—which renders them easily fermentable and perishable. Cold storage of the unpickled cheese is not employed for technical reasons. At storage temperature of 40–65° F., the cheese shrinks owing to whey drainage, dries as a result of evaporation, and some undesirable fermentations set in—starting from the surface and penetrating into the cheese mass—resulting in faulty flavours such as bitterness or fruitiness. At lower temperature ranges cheese may be frozen, ripening is hindered or stopped, undesirable flavours may be developed and texture may become crumbly on thawing. Cold storage of the tinned or barrelled pickled cheese is, however, a

common commercial practice. The pickling brine surrounding the cheese has a 'cushioning' effect which helps the cheese to retain its body and texture. The brine used for pickling Domiati cheese is usually prepared from whey expelled in the drainage process. Whole or separated milk containing the same concentration of salt as in the original cheese milk is sometimes used. Cheese for pickling is arranged in layers in suitable tins, and completely covered with brine. Finally, the tins are soldered and stored in a cold place to avoid 'blowing' caused by microbial fermentations, which may cause spoilage of the cheese and necessitates resoldering.

The packing of cheese in air-tight containers results in a fermentation, mainly anaerobic, and also excludes external contamination, vermin and insects.

Ripening period

Domiati cheese may be consumed fresh, but may be ripened for 4–8 months or longer. (In the latter case, this is usually done domestically in earthenware vessels.) There is no recognized ripening period which yields prime-quality cheese.

Yield

In Domiati, as in other types of cheese, yield varies with the composition of the cheese milk and the amount and composition of the whey drained.

Table 2 serves to illustrate the effect of the kind of milk used and percentage of salt added to the milk on the yield of fresh Domiati cheese.

Table 2. *The effect of concentration of added salt on the weight of fresh Domiati cheese obtained from* 100 *lb. of milk*

Kind of milk	Total solids (%)	Yield of cheese per 100 parts by weight of milk				
		+0% salt	+2·5% salt	+5% salt	+7·5% salt	+10% salt
Cows'	12·29	11·18	13·08	18·84	24·71	27·75
Buffaloes'	15·94	17·25	20·33	28·49	32·72	36·50

Chemical composition

This varies widely, and Table 3 gives the average percentages of the main constituents of good-quality whole-milk Domiati cheese.

Table 3. *Average composition of Domiati cheese*

	Moisture (%)	Total solids (%)	Fat (%)	Salt (%)
(1) Cows' milk:				
(*a*) Fresh cheese	59±4	41±4	18±2	4·5±0·5
(*b*) Ripened cheese (after 4–6 months)	55±3	45±3	20±2	4·9±0·6
(2) Buffaloes' milk:				
(*a*) Fresh cheese	55±4	45±4	24±3	4·5±0·5
(*b*) Ripened cheese (after 4–6 months)	52±3	48±3	25±3	4·8±0·6

II. EFFECT OF SALT CONCENTRATION UPON THE MANUFACTURE AND PROPERTIES OF DOMIATI CHEESE

It has been mentioned in Part I that Domiati is a soft cheese characterized mainly by the addition of a relatively large amount of salt to the milk prior to the addition of rennet. A study of some of the most important effects of this practice was considered to be of scientific and practical value.

The following points were investigated:

(1) Time of coagulation.
(2) Rate of whey drainage.
(3) Moisture retention in cheese.
(4) Acidity of cheese and whey.
(5) Fat content of cheese and whey.
(6) Chlorine distribution in cheese and whey.
(7) Nitrogen distribution in cheese and whey.
(8) Yield of cheese.

EXPERIMENTAL

The investigations were carried out on bulk cows' and buffaloes' milk. The required amounts of salt (2·5, 5, 7·5 and 10%) were added to the milk, an unsalted sample from each milk being used as a control. Every experiment was carried out ten times, and average values have been used in the discussion which follows.

Salt used in these experiments was of the best available quality. Its analysis was as follows:

	%
Sodium chloride	97·20
Sodium carbonate	0·37
Magnesium chloride	0·86
Magnesium sulphate	0·30
Calcium sulphate	0·34
Water	0·74
Nitrates	Nil
Total	99·81

The conditions of coagulation were chosen to simulate, on a small scale, those encountered in Domiati cheese-making. The experimental method followed was similar to that used by Rowland & Soulides (5) for Cheddar cheese, but with some modifications to suit the manufacturing process for Domiati cheese.

The required amounts of salt were dissolved in 100 ml. of milk placed in a glass beaker 7·5 cm. diameter and 8 cm. high. The beaker was covered and placed in a water-bath regulated at $35 \pm 0{\cdot}5°$ C., with the level of water 1·5 cm. above that of the milk. When the temperature of the milk reached 35° C., the beaker was agitated gently whilst 1 ml. of freshly dissolved Hansen's powdered rennet (2 g./100 ml. of water) was added. The desired coagulation was obtained when the curd separated cleanly from the sides of the beaker when pressed with a spatula, without any noticeable movement of coagulum particles. The curd was then poured into a cylindrical metal mould (10 cm. in diameter and 5 cm. in height) with a bottom having 20 holes to allow for whey drainage. The mould was lined with muslin cloth, and was mounted on a glass funnel which led the whey to a covered 100 ml. graduated measuring cylinder. The curd was left to drain for 24 hr., and the resulting fresh cheese and whey were taken for analysis. This procedure was satisfactory, since very little whey drainage occurred after 24 hr. Laboratory temperature varied between 25 and 35° C. during the experiments.

ANALYTICAL METHODS

Sampling

The milk used was well mixed and a suitable quantity taken for analysis. In the case of whey, the whole of the drained liquid was used as a sample. In the case of cheese, the

whole mass was taken as a sample, allowed to dry in a warm air current at 28–30° C., and the amount of moisture lost in drying was determined. The dried cheese was then ground in a mortar and stored in an air-tight sample jar in a refrigerator.

Chemical analysis of milk

Total solids were estimated by drying at 100° C. for 3 hr.

Acidity was determined in the usual way by titrating with N/9 NaOH, using phenolphthalein as an indicator.

Fat. Gerber's original method was used.

Specific gravity was estimated by means of a previously tested Quevenne lactometer observing the usual precautions and temperature corrections.

Chlorides. Davies's method (6) was followed, with minor modifications in the case of salted milk which had to be diluted in a volumetric flask and a sufficient quantity of silver nitrate added to effect precipitation of the chlorides.

Total protein and casein nitrogen were estimated by the semi-micro-Kjeldahl method modified by Rowland (7). As the nitrogen content of buffaloes' milk is greater than that of cows' milk, an increased amount of acid (30 instead of 25 ml. of 0·02 N sulphuric acid) was put into the receiving flask from buffaloes' milk.

Analysis of whey

The methods used for the determination of total solids, acidity, chlorides, total and protein nitrogen were the same as those used for milk. Specific gravity at 60° F. was estimated by a Westphal balance. Fat was determined by the Gerber 'Neusal' method, using the special whey butyrometers graduated from 0 to 1·5.

Analysis of cheese

For the determination of total solids the British Standards Institution (B.S.I. (8*a*)) sand method was used. For titratable acidity the B.S.I. method (8*b*) for soft cheese was adopted. The fat content of cheese was estimated in apparatus 1 B of the B.S.I. (8*c*), employing digestion with hydrochloric acid and removal of the fat with the extracting mixture specified.

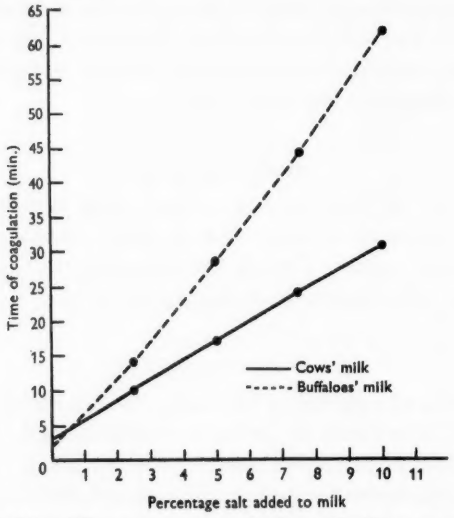
Davies's method (6) was followed for the determination of chlorides in cheese, with some modification in the amount of cheese and silver nitrate used due to the higher salt content of the cheese studied.

For total nitrogen, Rowland's method (7), slightly modified, was adopted. About 0·5 g. of the prepared sample was weighed into a Kjeldahl flask and wetted with 10 ml. of distilled water. Digestion and distillation followed and the ammonia was received in an excess (80 ml.) of 0·02 N sulphuric acid. The excess was titrated with 0·02 N sodium hydroxide.

Soluble nitrogen was determined as follows: about 5 g. of the prepared sample was mixed with dry digested sand and a little warm distilled water (about 20 ml.) at 50° C. The mixture was well stirred and left to settle. The clear liquid was filtered by suction through well-washed asbestos-wool. The extraction and filtration were repeated until about 200 ml. had been collected. The liquid was then transferred to a volumetric flask and made up to 250 ml. 25 ml. of the filtrate were taken and the nitrogen content determined by Rowland's method (7).

(1) *Time of coagulation*

The addition of an appreciable amount of salt to milk delays its coagulation by rennet. This fact is known to Domiati cheese-makers, who increase the amount of rennet added to counterbalance the retarding effect of salt. Several references confirm this observation. Thus, Sammis (4(*b*)) mentioned that the addition of enough salt to check bacterial growth will also delay rennet coagulation of milk. Heinemann (9) found that small quantities of the chlorides of alkalis accelerate rennin action, sodium chloride having this accelerating effect up to 0·9% concentration. Thom & Fisk (10) state that the soluble salts of ammonia, sodium and potassium retard or prevent rennet coagulation. Van Slyke (11) gave sodium chloride as an example of substances which if present in certain amounts retard rennet coagulation of milk. Lorcher (12) studied the influence of various cations on rennin coagulation of milk, using chlorides of elements. The monovalent ions, lithium, sodium and potassium, show no rise with increasing concentration, but generally depress the rate of rennin coagulation of whole milk.



Text-fig. 4. Effect of adding different percentages of sodium chloride on coagulation time of milk.

Further to elucidate this point, and to compare the behaviour and time of coagulation of cows' and buffaloes' milk containing the same amount of salt, ten experiments were carried out according to the procedure previously mentioned.

The averages of the results obtained are shown in Text-fig. 4. The data indicate that with higher percentages of salt, time of coagulation increased and a softer curd was formed. They also show that salted buffaloes' milk always took longer to coagulate than did cows' milk containing the same amount of salt. Unsalted buffaloes' milk, however, contrary to expectation, gave a shorter coagulation time than did cows' milk. The explanation of these findings is rather obscure owing to lack of precise information regarding the reactions associated with rennet coagulation. Possible explanations may be:

(1) Considering rennin as an acid protein of the globulin type, it (like other proteins) may be salted out of solution by sodium chloride (13, 14(*a*)), and thus its action is retarded by increased amounts of salt, resulting in a longer time of coagulation.

(2) On the principle of base exchange, the addition of sodium chloride to calcium caseinate causes the replacement of the calcium ions by sodium ions with the formation of sodium caseinate or sodium-calcium caseinate complex and calcium chloride as is believed to occur in the preparation of soft curd milk by the zeolite treatment, addition of sodium citrate of phosphate (14(*b*)) and (15).

According to Bosworth (16) the reactions taking place may be represented as follows:

$$\text{Ca caseinate} + \text{NaCl} \rightleftharpoons \text{Na or NaCa caseinate} + \text{Ca Cl}_2.$$

As proposed by Van Slyke (17) the reactions may be expressed as follows:

$$\text{Ca} \begin{cases} \text{Caseinate} + 2\text{NaCl} \rightleftharpoons 2\text{Na caseinate} + \text{CaCl}_2 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \text{Caseinate} \qquad\qquad\qquad\qquad\quad \text{Soluble} \\ \quad \downarrow \\ \text{Insoluble} \end{cases}$$

With the addition of rennet, the sodium or sodium calcium caseinate is changed to the corresponding paracaseinate. This paracaseinate is more hydrophilic in nature with higher absorption capacity, and gives a softer coagulum in the presence of soluble calcium salts than does the normal calcium paracaseinate.

(3) Both the foregoing factors may operate together.

The apparently slower rate of coagulation of salted buffaloes' milk compared with salted cows' milk may be due to the softer curd formed from the former which obscures the first visible signs of coagulation.

On the other hand, the slower rate of coagulation of unsalted cows' milk compared with buffaloes' milk may be due to the fact that buffaloes' milk contains more solids (including casein) and yields a noticeably firmer curd than does cows' milk; with increasing firmness of curd earlier recognition of normal signs of coagulations is possible.

(2) *Rate of whey drainage*

In the manufacture of Domiati cheese, it is well known that the rate of whey drainage is decreased as increased salt is added to the milk. To obtain quantitative data ten experiments were carried out and the averages of the results obtained are given in Table 4.

The volume of whey drained from buffaloes' milk curd after 24 hr. was always less than from cows' milk curd containing the same percentage of salt. This may be due to the higher percentage of water in cows' milk and to the more pronounced effect of salt in delaying drainage of buffaloes' curd.

With 10% salt, it was noticed that in the early stages whey drained from buffaloes' milk curd exceeded that from cows' milk curd. This increase, however, was temporary and lasted only for the first 90 min. The early excess of buffaloes' curd whey with higher salt concentration was primarily due to the softness of the curd resulting in the loss of some curd constituents into the milky whey.

The data also indicate that the maximum amount of whey drained after 24 hr. was obtained with 5% salt in the case of cows' curd and with 2·5% in buffaloes' curd. Thus, it would appear that the addition of small quantities of salt hastens the drainage process, the concentration of salt required to enhance drainage varying with the kind of milk used. In the case of buffaloes' milk soft curd formation with the addition of salt is more pro-

nounced and less salt is required to speed drainage than in cows' milk. Above these values, the volume of whey collected after 24 hr. decreased with increased salt concentration.

Table 4. *Effect of adding different percentages of salt on the speed of drainage and total amount of whey drained in ml. from* 100 *ml. of milk*

	Period of drainage														
	30 min.					60 min.					90 min.				
% salt added	0	2·5	5	7·5	10	0	2·5	5	7·5	10	0	2·5	5	7·5	10
Cows' milk	28	19	13	11	9	33	24	17	14	12	37	28	21	18	16
Buffaloes' milk	19	12	7	8	10	25	18	13	13	14	28	22	17	17	17

	Period of drainage									
	120 min.					24 hr.				
% salt added	0	2·5	5	7·5	10	0	2·5	5	7·5	10
Cows' milk	40	32	24	21	19	55	61	67	63	58
Buffaloes' milk	32	26	22	20	18	52	61	57	54	49

The average maximum and minimum temperatures during the experiments were 32 and 25° C. respectively.

(3) *Moisture retention in cheese*

It has been shown (p. 320) that the addition of salt retards the coagulation of milk by rennet and that the retarding effect increases with increased amounts of salt and results in a soft curd. That this softness of the curd is due to the incorporation of more moisture in the cheese is evident from Text-fig. 5. The results obtained (Text-fig. 6) indicate that the amount of whey drained decreased with increased additions of salt to the milk. These results are in agreement with those of Koestler & Peterman (18), who reported that milk slow to coagulate expels only very slowly a whey rich in fat.

The results also show that the water content of both whey and cheese produced from cows' milk was always higher than that produced from buffaloes' milk with the same percentage of salt, as would be expected from the larger amount of water in the original cows' milk which contained 87·18% moisture as compared with 84·12% in buffaloes' milk.

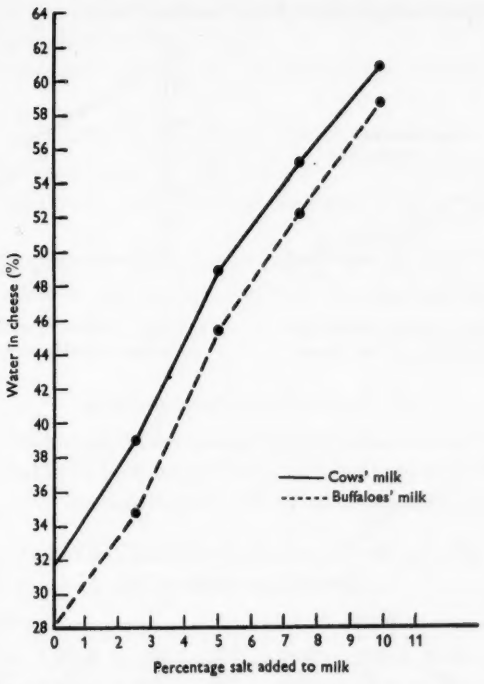
(4) *Acidity of cheese and whey*

The results obtained (Text-figs. 7 and 8) show that:

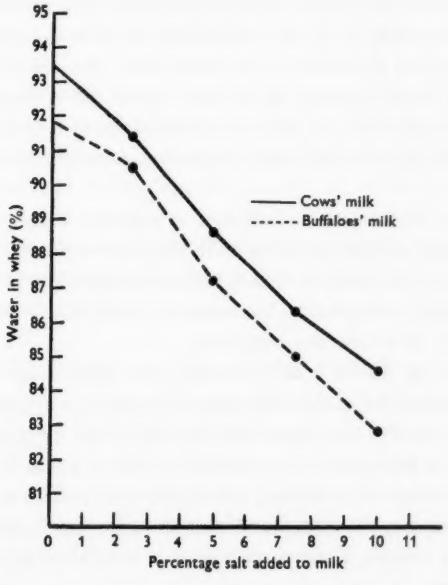
(*a*) Increased addition of salt to milk was accompanied by a decrease in the acidity developed in the cheese and whey. This is apparently due to the inhibitory action of salt on the lactic acid bacteria. Such a retarding effect has been reported by numerous investigators. McDowall & Whelan's experiments (19) show that while the addition of 1% always and 2% frequently stimulated the growth of lactic acid bacteria, 3% usually and 5% salt regularly inhibited their action. Total inhibition was approached at any concentration greater than 6%.

(*b*) The acidity, with similar amounts of salt, developed more slowly in buffaloes' milk than in cows' milk products. This may be due to the higher buffer action of buffaloes' milk and lower moisture content of buffaloes' milk products.

(*c*) The acidity of cheese was noticeably higher than that of the corresponding whey for all salt concentrations. This is in accord with Orla-Jensen's statement (20) that when milk is curdled with rennet most of the bacteria from the milk remain in the curd, the whey gaining acidity mainly by the movement of acid from the curd.

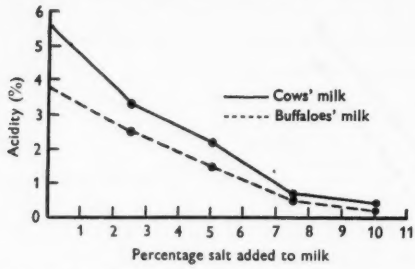


Text-fig. 5. Effect of adding different percentages of sodium chloride on the water content of the cheese.

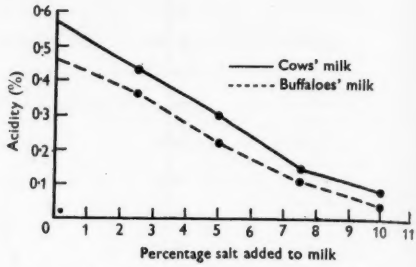


Text-fig. 6. Effect of adding different percentages of sodium chloride on the water content of the whey.

The acidity figures for whey refer to samples examined after 24 hr. drainage, and consequently high values were obtained from unsalted milks.



Text-fig. 7. Effect of adding different percentages of sodium chloride on the acidity of the cheese.



Text-fig. 8. Effect of adding different percentages of sodium chloride on the acidity of the whey.

(5) *Fat content of cheese and whey*

As fat is one of the main constituents of cheese, every precaution is taken to minimize its loss in whey. Data showing the effect of salting on the loss of fat are given in Table 5, which also shows the percentage of fat in the cheese obtained.

Table 5. *Effect on the fat content of whey and fresh cheese of adding different percentages of salt*

		Whey					Cheese				
Milk	% of fat	0% salt	2·5% salt	5% salt	7·5% salt	10% salt	0% salt	2·5% salt	5% salt	7·5% salt	10% salt
Cows'	3·6	0·08	0·10	0·14	0·19	0·24	32·79	27·60	20·74	16·78	14·26
Buffaloes'	6·4	0·13	0·16	0·20	0·29	0·48	39·03	31·08	25·81	20·62	17·23

The data show that although the loss of fat in buffaloes' was greater than in cows' whey, yet it was not strictly proportional to the percentage of fat in the original milks. This is in agreement with Van Slyke & Price (11 (*b*)), who state that 'the loss of fat in cheese-making is quite independent of the amount of fat in milk. Even when cream is added to normal cows' milk to an extent sufficient to raise the fat content to 7 or 8%, the increased loss of fat, though considerable, is not necessarily greater in proportion to the increase of fat in milk.'

The higher percentage of fat lost in buffaloes' whey may be attributed to the greater number of large-size fat globules in buffaloes' milk than in cows' milk (21). Thom & Fisk (10 (*b*)) and Eckles, Combs & Macy (22) mention that in milk with large-size fat globules, appreciable creaming takes place before coagulation has advanced sufficiently to check the movement of the rising fat and lock it within the coagulum.

The figures presented in Table 5 indicate that the percentage of fat in the cheese increases with the increase of fat in the milk used. This again is in accord with Van Slyke & Price (11 (*c*)). The data in Table 5 also show that increasing the amount of salt added to the milk led to an increase of fat in the whey obtained. This is again in agreement with Van Slyke & Price (11 (*c*)), who state that among the numerous conditions causing an increased loss of fat in cheese-making is the addition of salt to milk, which interferes with complete coagulation of casein by rennet. Thus, a milk slow to coagulate expels, only very slowly, a whey rich in fat.

To summarize, the results indicate that the loss of fat in buffaloes' whey was higher than in cows' whey, the loss being particularly evident with higher levels of salting owing to:

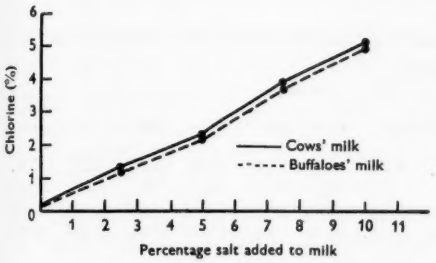
(*a*) the softer curd of salted buffaloes' milk,

(*b*) the higher percentage of fat and the greater number of large-size fat globules in buffaloes' milk (21).

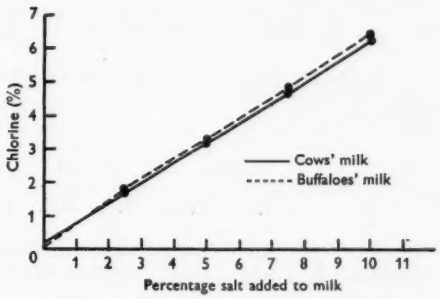
(6) *Chlorine distribution in cheese and whey*

A knowledge of the distribution of salt between the final cheese and whey from the salted milk is most valuable in fixing the right amount of salt to be added to the cheese milk.

In these experiments the average chlorine contents of unsalted cows' and buffaloes' milk were 0·093 and 0·082% respectively. Previous determinations by the authors have given similar results.



Text-fig. 9. Effect of adding different percentages of sodium chloride on the chlorine content of the cheese.



Text-fig. 10. Effect of adding different percentages of sodium chloride on the chlorine content of the whey.

The chlorine distribution with varying percentages of salt added to milk is shown in Text-figs. 9 and 10. These results indicate that with increasing additions of salt to the milk, the chlorine content increased in both the cheese and the whey. Salted buffaloes' milk always yielded a whey with a higher chlorine content than an equally salted cows' milk. Since the salt in water concentration is higher in salted buffaloes' milk, this might be expected if cows' and buffaloes' milk behave similarly during drainage.

The difference between the percentage of chlorine in buffaloes' and cows' whey was nearly constant. This may be explained by the higher salt-in-water concentration in the buffaloes' milk. With approximately the same amount of salt added to both cows' and buffaloes' milk, the percentage of salt retained in cows' milk cheese was higher than in buffaloes'. This is probably due to the greater amount of moisture in cows' than in buffaloes' cheese.

(7) *Nitrogen distribution in cheese and whey*

Since the nitrogenous compounds in milk have a major influence upon the chemical and other properties of cheese and whey, the effect of varying additions of salt to the milk upon the nitrogen distribution in cheese and whey was investigated. Results presented in Tables 6 and 7 show that whey produced from buffaloes' milk was always higher in total and protein nitrogen than that from cows' milk; higher percentages of both are present in the former milk.

The percentage of total nitrogen in whey decreased as the percentage of salt dissolved

in the milk increased. The percentage of protein nitrogen in whey rose to a maximum with 2·5% salt and decreased at higher percentages, but was still higher than in the unsalted whey. This probably resulted from the increased solubility of proteins in dilute brine and their insolubility in strong brine in accordance with Orla-Jensen's findings (20 (*b*)).

Table 6. *Effect of adding different percentages of salt on the nitrogen distribution of cheese*

	Milk Nitrogen distribution			Cheese									
				0% salt		2·5% salt		5% salt		7·5% salt		10% salt	
	T.N. %	P.N %	S.N. %	T.N. %	S.N. %	T.N. %	S.N. %	T.N. %	S.N. %	T.N. %	S.N. %	T.N. %	S.N. %
Cows'	0·48	0·452	0·100	2·71	0·146	2·43	0·059	2·03	0·037	1·79	0·029	1·65	0·019
Buffaloes'	0·60	0·556	0·136	2·37	0·110	2·09	0·045	1·85	0·028	1·65	0·020	1·52	0·016

Note. T.N. = total nitrogen; P.N. = protein nitrogen; S.N. = soluble nitrogen.

Table 7. *Effect of adding different percentages of salt on the nitrogen distribution of whey*

	Milk		Whey									
			0% salt		2·5% salt		5% salt		7·5% salt		10% salt	
	T.N. %	P.N. %	T.N. %	P.N. %	T.N. %	P.N. %	T.N. %	P.N. %	T.N. %	P.N. %	T.N. %	P.N. %
Cows'	0·48	0·452	0·073	0·027	0·070	0·033	0·066	0·031	0·064	0·030	0·061	0·029
Buffaloes'	0·60	0·556	0·097	0·042	0·192	0·047	0·084	0·045	0·081	0·044	0·077	0·042

Note. T.N. = total nitrogen; P.N. = protein nitrogen.

The difference in the percentage of protein nitrogen of both wheys was nearly constant in the unsalted and salted wheys. This was probably due to the similar nature of the proteins in both milks (14 (*b*)). On account of calculating the percentages given in Table 6 on a total and not salt-free basis, the total nitrogen content of both unsalted cows' and buffaloes' milk cheese appeared to be higher than their respective salted cheese. Unsalted cheese contained a markedly higher percentage of soluble nitrogen, which decreased with increasing additions of salt to the milk. This was probably due to the retardation of the activity of proteolytic organisms by the salt.

(8) *Yield of cheese*

Table 8. *Effect of adding different percentages of salt on the yield of cheese*

Milk	Total solids (%)	Yield of cheese (g./100 ml. of milk)				
		0% salt	2·5% salt	5% salt	7·5% salt	10% salt
Cows'	12·29	11·18	13·08	18·84	24·71	27·75
Buffaloes'	15·94	17·25	20·33	28·49	32·72	36·50

Data presented in Table 8 show that the yield from buffaloes' milk was always higher than that from cows' milk containing the same amount of salt. This is due to the fact that buffaloes' milk contains the higher casein and fat content. The difference between the two yields was at its highest with 5% salted milk.

The yield from both milks increased with increasing additions of salt, being most pronounced between 2·5 and 5% salt in the case of buffaloes' milk and between 2·5 and 7·5% salt in cows' milk. Salt affects the yield, in this case in two ways:

(1) Directly by being incorporated into the cheese.

(2) Indirectly by causing formation of a soft curd which retains a greater percentage of moisture, causing an increase in yield.

SUMMARY

In the first part of this study, brief notes are given on the better known varieties of the soft pickled group of cheese which the authors believe to be best suited to warm climatic conditions.

Domiati cheese, a variety of this group, characterized by the addition of an appreciable amount of salt to the milk prior to rennetting, is described in detail. An outline of the steps of its manufacture, the pickling process, and the effect of the amount of salt added to the milk on the composition of the resulting cheese is given.

In the second part the effects of varying additions of salt to both cows' and buffaloes' milk on rennet coagulation, rate of whey drainage, chemical composition and yield of cheese and whey were investigated.

(1) It has been shown that with increased additions of salt, coagulation by rennet is retarded, a softer curd is formed, the rate of whey drainage is decreased and the yield of cheese is increased by increased retention of water.

(2) Acidity development is also retarded and fat losses in the whey are increased, particularly at the higher salt concentrations.

(3) The chlorine content of buffaloes' milk was found to be lower than that of cows' milk. The wheys produced from both cows' and buffaloes' cheese milks had a slightly higher chlorine content than their respective milks.

With approximately the same amount of salt added to both cows' and buffaloes' milk, the percentage of salt retained in cows' milk cheese was higher than it was in buffaloes' milk cheese.

(4) When 2·5% of salt was added to both cows' and buffaloes' milk the protein nitrogen content of the wheys produced averaged 0·033 and 0·047% respectively. These values decreased progressively with increments of salt reaching the values of 0·29 and 0·042% respectively for 10% salt content. This indicates that the solubility of protein was highest at the lowest salt concentration used in these experiments.

(5) Differences observed between the behaviour of cows' and buffaloes' milk, both salted and unsalted, have been related to the differences in their chemical composition.

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REFERENCES

(1) DOANE, G. F., LAWSON, H. W. & MATHESON, K. J. (1932). *Bull. U.S. Dep. Agric.* no. 608.

(2) EGYPTIAN MINISTRY OF AGRICULTURE (1946). *Records Department of Agricultural Economics and Statistics.*

(3) DAVIS, J. G. (1945). *Food Industries Manual*, p. 546. London: Leonard Hill Ltd.

(4) SAMMIS, J. L. (1942). *Cheese Making*, pp. 43, 57. Madison, Wisc.: The Cheese Maker Book Co.

(5) ROWLAND, S. J. & SOULIDES, D. (1942). *J. Dairy Res.* **15**, 86.

(6) DAVIES, W. L. (1932). *Analyst*, **57**, 79.

(7) ROWLAND, S. J. (1938). *J. Dairy Res.* **9**, 30.

(8) British Standards Institution (1938). No. 770, (*a*) p. 7, (*b*) p. 13, (*c*) p. 9.
(9) Heinemann, P. G. (1921). *Milk*, p. 94. London: Sanders Co. Ltd.
(10) Thom, C. & Fisk, W. W. (1925). *The Book of Cheese*, (*a*) p. 35, (*b*) p. 8. New York: Macmillan Co.
(11) Van Slyke, L. L. & Price, W. V. (1941). *Cheese*, (*a*) p. 120, (*b*) p. 52, (*c*) p. 53. New York: Orange Judd Publ. Co. Inc.
(12) Lorcher, G. (1897). Cited from Davies, W. L. (14). *The Chemistry of Milk*, 2nd ed. p. 338. London: Chapman and Hall Ltd.
(13) Ling, E. R. (1946). *Dairy Chemistry*, 2nd ed. 1, p. 145. London: Chapman and Hall Ltd.
(14) Davies, W. L. (1939). *The Chemistry of Milk*, 2nd ed. (*a*) p. 329, (*b*) p. 115. London: Chapman and Hall Ltd.
(15) Sommer, H. H. (1946). *Market Milk and Related Products*, 2nd ed. p. 521. Madison, Wisc.: Author
(16) Bosworth, A. W. (1916). *Tech. Bull. N.Y. (Geneva) agric. Exp. Sta.* no. 53.
(17) Van Slyke, L. L. (1928). *Tech. Bull. N Y. (Geneva) agric. Exp. Sta.* no. 139, p. 31.
(18) Koestler, G. & Peterman, R. (1936). *Landw. Jb. Schweiz*, **50**, 103.
(19) McDowall, F. H. & Whelan, L. A. (1933). *J. Dairy Res.* **4**, 42.
(20) Orla-Jensen, S. (1931). *Dairy Bacteriology*, 2nd. ed. (*a*) p. 142, (*b*) p. 143. London: Churchill.
(21) Zein El-Abideen (1945). Ph.D. Thesis, Fouad-El-Awal University, Giza, Egypt.
(22) Eckles, C. H., Combs, W. B. & Macy, H. (1945). *Milk and Milk Products*, p. 24. New York: McGraw Hill Co.

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430. STUDIES OF THE NATURE OF SUBJECTIVE JUDGEMENTS IN CHEESE-GRADING

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INTRODUCTION

In grading Cheshire cheese, assessments are made of the 'body', 'texture', 'crumbliness', flavour and overall quality. In commercial grading in this country (England) it is not usual to apply a scoring system to these various characteristics, although, in judging cheese for show purposes, points would be assigned to each item. American practice, at least according to the literature, suggests that score cards are in general use for each particular dairy product, so that the various items may be assessed in quantitative terms. For experimental purposes, where it is required to assess the effect of specific variations in conditions of manufacture or storing of the cheese, or to assess the relation between instrumental tests and the judgements of graders and others, some quantitative form of expression of these judgements is essential. However, it has to be borne in mind that the process of forcing the judgement into an arbitrarily selected numerical scale may lead to a certain amount of distortion and may not be entirely satisfactory. Modifications of procedure desirable in the interests of statistical planning may also be unacceptable, in view of the dislocation they may cause to the ingrained habits of the professional food-grader.

In the present paper selected data, obtained during the course of a systematic experiment on the relationship between subjective judgements of the essential characteristics of Cheshire cheese and a series of rheological measurements (flow or deformation under pressure), are described. Emphasis will be placed mainly on the consistency and the discrimination of the subjective judgement, and on individual differences in these two items. Most of the data which are reported here were obtained during intervals in the main investigation, when, owing to shortage of milk, the continuous supply of cheese ceased temporarily. The only objective measurement, which is used for comparison purposes, is the penetration of a loaded sphere into the surface of the cheese in a period of 10 sec. This instrument, the Ball Compressor, which has been described in detail elsewhere (1), appears to provide one of the most useful, single, objective measurements yet available.

EXPERIMENTAL PROCEDURE

The procedure was similar to that used in a previous study (2), and the experiments were, in the main, a continuation of the earlier work. Instead of using a standard score card, the individuals who took part were asked to rate each cheese on a seven-point scale for each of the items set out in Table 1. In each case it was understood that the centre point of the scale was to represent the normal, or average, cheese. Judgements were recorded on a specially prepared form with space for descriptive items and comments, where necessary. The usual procedure was to assess all the cheese in a given group for firmness on the outside, and then to work through the list of remaining characteristics for each

cheese separately. Strictly speaking, it might be desirable to obtain the separate assessments in carefully randomized orders. This might have been done both in respect of the cheese and the items assessed. In addition, it might have been desirable to permit the assessor to see only one item at a time and to withdraw the record card from him immediately the appropriate numerical value had been entered. This was not done, and in spite of changing the marking numbers and rearranging the cheese it was impossible to prevent recognition that each cheese was being presented several times and the previous rating might, occasionally, have been remembered. However, it seems to the writers that such recognition is, inevitably, one of the results of accumulated experience and skill.

Table 1. *Selected ratings made on each cheese*

	Rating		
	1	4	7
Firmness (*W*). Assessed by pressing the thumb into the cheese (*W* stands for whole)	Very firm	Average	Very soft
Firmness (*P*). Assessed by pressing the thumb into a sample from within the cheese (*P* stands for plug)	Very firm	Average	Very soft
Flavour	Excellent	Average	Very poor
Overall quality	Excellent	Average	Very poor

In the first experiment, five cheese of the same age, selected from factory production, were presented for judgement to each of three individuals, on nine separate occasions. The cheese were all 2 weeks old and were not widely different in overall quality (i.e. from a commercial point of view they were all Grade I). A random order of presentation was used, a different order for each subject and each occasion, but the identifying numbers remained unchanged. The experimental data will be discussed in relation to those from the second experiment which arose out of the first. In the first experiment it became evident, even on mere inspection of the data, that the professional grader was not differentiating between the cheese. Therefore, the later experiment was planned in such a way that certain of the cheese were clearly different. In fact, two cheese were selected from each of three distinct batches. These batches are subsequently designated A, B and C. Further modifications in the second experiment were made. The number of replicate assessments on separate occasions was reduced to four. Also the distinguishing marks were selected, according to the suggestions made by Hening (3), in such a way as to have no meaning or suggestion in terms of the ratings. The marking system involved changing the cards from one cheese to another on successive occasions, in addition to randomizing the order in which the cheese were tested.

THE SUBJECTS, OR CHEESE TESTERS

Three individuals, X, Y and Z, took part in both experiments. All three had previously been rating cheese, using the same general scheme, for several weeks. X was concerned with the organization of the research programme, in addition to taking part in the objective measurements on the various cheese, in which she was assisted by Y. Z was a professional grader of long experience. Two additional individuals took part in the second experiment. One, E, was the experimenter in the present series. Although intensely interested in the outcome of the experiments, he had had little recent practice using the rating scale technique. The other, α, was an interested visitor who should be described as

a professional rheologist, i.e. his particular interests lay in the mechanical testing of the cheese.

TREATMENT OF THE DATA

The average ratings for each individual on each cheese have been tabulated for the selected characteristics. In addition, the following quantities, calculated by applying analysis of variance to the data for each individual, have been included:

(1) The mean square error (M.S.E.).

(2) The number of degrees of freedom on which this is based (D.F.).

(3) The ratio of the variance contributed by differences between cheese (Var. C) to the error term (Var. E).

Since the assumptions behind the use of significance tests are not necessarily satisfied by individual ratings, no significance levels have been assigned. The technique has been used mainly for descriptive purposes. The mean square error gives an indication of the *consistency of judgement*. The ratio of the variance contributed by differences between cheese to the error term gives an indication of *the effectiveness of discrimination*.

DISCUSSION OF RESULTS

The most satisfactory method appears to be first to review the salient characteristics of the separate experiments, and then to relate them.

(*a*) *Experiment* 1*

The judgements of firmness both on the outside of the whole cheese and on the plug show that *consistency* of judgement of the three subjects is roughly comparable, whereas there are marked individual differences in *discrimination*. X shows pronounced differentia-

Table 2. *Experiment* 1

(*a*) Firmness (*W*): mean of nine replicate judgements

Grader ...	X	Y	Z
Cheese			
1	2·3	2·4	1·1
2	2·9	2·2	1·0
3	4·9	3·0	1·3
4	3·9	2·4	1·3
5	2·2	1·9	1·0
M.S.E.	0·16	0·16	0·12
D.F.	40	40	40
Var. C/Var. E	72·0	9·2	2·1

(*b*) Firmness (*P*): mean of nine replicate judgements

Grader ...	X	Y	Z
Cheese			
1	2·4	3·1	2·8
2	2·7	3·2	2·8
3	4·4	3·4	2·7
4	3·4	3·3	2·6
5	1·9	2·4	2·4
M.S.E.	0·18	0·14	0·23
D.F.	32	32	44
Var. C/Var. E	48·0	10·0	0·8

(*c*) Flavour: mean of nine replicate judgements

Grader ...	X	Y	Z
Cheese			
1	3·2	2·7	3·0
2	3·1	2·7	3·0
3	5·0	2·7	3·0
4	3·9	3·0	3·0
5	3·2	2·6	3·0
M.S.E.	0·22	0·23	0·00
D.F.	40	40	—
Var. C/Var. E	25·6	1·1	—

(*d*) Overall quality: mean of nine replicate judgements

Grader ...	X	Y	Z
Cheese			
1	3·4	2·3	3·0
2	3·6	2·4	3·0
3	6·1	2·3	3·0
4	3·4	2·6	3·0
5	3·4	2·2	3·0
M.S.E.	0·26	0·25	0·00
D.F.	40	40	—
Var. C/Var. E	11·9	0·6	—

* See Table 2.

tion between the cheese, Y slight differentiation, Z (the professional grader) shows no differentiation. At the same time, according to the average scale value, while X rates the cheese as firm on the outside as on the plug, the other two show a change in judgement towards a softer assessment on the objectively softer plug. This suggests that only X was in fact judging firmness strictly in relation to the average for a normal cheese.

The professional grader (Z) shows complete consistency but no differentiation in the flavour and overall quality of the present group of cheese. The other two individuals appear to be slightly less consistent in assessing flavour and overall quality than they were in assessing firmness. Only X, however, shows any clear differentiation between the cheese.

The absence of differentiation by the professional grader between the present cheese raises several interesting questions. Since, as has been mentioned earlier, the cheese were all Grade I, commercially, it is believed that previous habits of judgement had a predominant influence. However, is the perfect consistency an artifact or has it any effective meaning? A partial answer to this question is probably forthcoming from the second experiment which will now be discussed.

(*b*) *Experiment* 2*

In assessing firmness, the professional cheese grader (Z) shows complete consistency, but no differentiation, although he again rates the sample from within as softer than the outside of the cheese. Of the remaining individuals X shows a particularly high level of discrimination and consistency in assessing firmness on the outside of the cheese. In general, X, Y and E show a decrease in both consistency and discrimination when assessing the firmness of the plug, as compared with firmness of the whole cheese. For α, the change appears to be in the opposite direction. Taking the discrimination and consistency together, the assessment suggests that judgements of firmness are more effective for the experienced group (X, Y) than for the casual group of assessors (E and α), although further investigation would be required to state conclusively that the effect is attributable to practice or training.

Assessments of flavour and quality show that in recording his judgement in this form, the professional grader shows no unusual consistency, when he finds it necessary to discriminate. Indeed, of the three regular participants, differences in consistency are small, but X and Y are clearly much more discriminating. In rating flavour, the visitor (α) shows an extraordinary lack of consistency, although this reverts to an expected value when assessing quality.

(*c*) *Comparison of the two experiments*

Although the changes of design and length of the two separate experiments make comparisons a little uncertain as to their origin, several striking features may be observed. The assessments for X and Y show a pronounced increase in differentiation when the sample of cheese covers a wider range of differences, although the changes for firmness (plug) are small. The professional grader, on the other hand, decides more emphatically that the cheese covering the wider range of firmness cannot be differentiated subjectively. His flavour and overall quality assessments change from perfect consistency with no discrimination to roughly average consistency (as compared with X and Y), but still very inadequate differentiation.

* See Table 3.

Table 3. *Experiment* 2

(*a*) Firmness (*W*): mean of four replicate judgements

Grader ...	X	Y	Z	E	α
Cheese					
A 1	2·7	3·7	2·0	2·7	2·7
A 2	1·0	2·0	2·0	1·2	2·0
B 3	6·0	6·2	2·0	7·0	5·7
B 4	6·0	6·2	2·0	6·2	6·2
C 5	4·0	4·2	2·0	3·0	4·7
C 6	4·2	5·0	2·0	3·2	4·0
M.S.E.	0·08	0·17	0·00	0·50	0·61
D.F.	18	18	—	18	15
Var. C/Var. E	179·6	63·5	—	39·5	18·2

(*b*) Firmness (*P*): mean of four replicate judgements

Grader ...	X	Y	Z	E	α
Cheese					
A 1	2·7	3·2	3·0	3·0	3·0
A 2	2·0	2·2	3·0	2·2	1·7
B 3	6·2	6·0	3·0	5·5	6·0
B 4	6·0	6·5	3·0	5·2	6·2
C 5	3·7	4·5	3·0	3·2	3·7
C 6	3·7	5·0	3·0	3·0	4·5
M.S.E.	0·28	0·64	0·00	0·68	0·40
D.F.	18	18	—	15	18
Var. C/Var. E	42·1	16·3	—	10·5	30·4

(*c*) Flavour: mean of four replicate judgements

Grader ...	X	Y	Z	E	α
Cheese					
A 1	2·5	3·2	4·2	2·7	4·0
A 2	2·2	2·0	4·0	2·5	3·5
B 3	7·0	7·0	5·2	5·7	4·2
B 4	7·0	7·0	5·2	5·2	5·5
C 5	4·0	4·2	4·2	3·5	3·2
C 6	3·5	5·0	4·0	3·2	2·5
M.S.E.	0·22	0·19	0·28	0·38	2·03
D.F.	15	18	18	18	18
Var. C/Var. E	82·7	85·2	5·0	19·1	2·0

(*d*) Overall quality: mean of four replicate judgements

Grader ...	X	Y	Z	E	α
Cheese					
A 1	3·0	3·2	4·2	2·7	4·0
A 2	2·7	2·0	4·0	2·5	3·2
B 3	7·0	6·2	6·0	6·2	5·2
B 4	7·0	6·5	6·2	5·7	6·0
C 5	4·2	4·2	4·0	3·5	3·5
C 6	4·0	5·0	4·0	3·2	3·0
M.S.E.	0·21	0·29	0·31	0·39	0·58
D.F.	15	18	18	18	18
Var. C/Var. E	67·6	41·8	14·8	26·1	13·4

Perhaps some suggestions for the possible source of these differences may be made. The highest differentiation and consistency is shown by X who, in addition to practice, is probably *looking for differences*, for she knows that only by differentiating to the maximum can the subjective judgement effectively be related to objective measurements. The cheese

grader, on the other hand, is *intended* to represent his profession and, in particular, current practice. In view of the existence of only three categories of cheese in professional (M.O.F.) grading it is hardly to be expected that a wide range of differentiation would normally be used. With regard to the inadequacy of the judgement of firmness, it should be stated that, in contrast to previous individuals, the cheese grader exerted only a very light pressure with the thumb and considered the assessment of firmness to be unimportant. It is possible that if an assessment of 'body' rather than 'firmness' had been requested, a different result might have been obtained. Although different sensory cues would have been used by the grader, in the ideas of the experimenters, there is a close relation between what they term 'firmness' and what those in the profession term the 'body' of a cheese.

Table 4. *A comparison between subjective ratings for firmness on the outside of the cheese, with objective measurements*

	Objective test	Subjective data				
		X	Y	Z		
Exp. 1: Var. C/Var. E	7·9	72·0	9·2	2·1		
Replications	5	9	9	9		
		X	Y	Z	E	α
Exp. 2: Var. C/Var. E	57·0	179·6	63·5	—	39·5	18·2
Replications	5	4	4	4	4	4

(*d*) *Comparison between subjective ratings for firmness and objective measurements*

In Table 4, data are given in which the subjective ratings for firmness and the objective measurements by means of the ball compressor are compared. The most suitable method of comparison is to tabulate the ratio (variance between cheese)/(variance attributable to error) for the subjective ratings for each individual and for the replicate measurements. Both sets of objective measurements involved five tests on each cheese, whereas the subjective ratings involved nine replications in the first experiment. Comparisons are restricted to assessments of firmness on the outside (top) of the cheese. In both experiments, for the individual previously described as 'looking for differences', the subjective discrimination appears to be more effective than the instrumental test. A direct visual comparison between the two sets of numbers suggests that the relation between these subjective assessments and the objective measurements is substantially linear.

MAIN CONCLUSIONS

The essential conclusions from the present investigation are:

(1) The professional grader when discriminating between a number of cheese shows no unusual degree of consistency.

(2) In general his discrimination covers a more restricted range than others. This may be the result of current commercial grading practice.

(3) His discrimination of firmness is negligible. This is not surprising in view of the way he 'thumbs' the cheese and his statement that he did not consider firmness, as such, to be important.

(4) The most effective differentiation was shown by X, who knew intimately the purpose of the present experiments and had considerable practice using the rating scale method. She might be termed as 'looking for differences'.

(5) The interested casual subjects in general showed less consistency than the regular or practised group.

(6) A comparison between subjective and objective (instrumental) estimates of firmness showed that for the individual who was termed 'looking for differences', the subjective form of discrimination was more effective than the objective measurements, under similar conditions.

The implications of the present study from a point of view of future planning are:

(*a*) Particularly where it is required that the subjects shall *look for differences*, this requirement should be stated explicitly, and a preliminary trial on the proposed group is most desirable.

(*b*) The present data suggest that the effectiveness of judgement is dependent upon the length of practice. For use as a subjective standard it is clearly desirable to familiarize the subject with the experimental technique and to allow a substantial amount of preliminary practice.

(*c*) Where detailed assessments of each cheese are required the number actually used (five or six) seemed to be reasonable.

(*d*) In general nine replications appeared rather excessive; four were finally considered to be adequate.

(*e*) As the professional grader showed no differentiation between the cheese for firmness, it would have been useful to have included an assessment for 'body'.

The writers are indebted to the Directors of United Dairies Ltd. for placing facilities at their disposal at the Ellesmere Creamery, and to the Manager and Staff of the Creamery for their co-operation during this study.

Certain aspects of the statistical planning of these experiments were discussed with Mr C. P. Cox. One of us (R. H.) wishes to acknowledge a grant from the Agricultural Research Council.

REFERENCES

(1) CAFFYN, J. E. & BARON, M. (1947). *Dairyman*, **64**, 345.
(2) HARPER, R. & BARON, M. (1949). *J. Dairy Res.* **16**, 363.
(3) HENING, J. C. (1948). *Tech. Bull. N.Y. St. agric. Exp. Sta.* no. 284.

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431. TWO SAMPLES OF 7-YEAR-OLD CHEDDAR CHEESE

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During the autumn of 1946, samples of two Cheddar cheese, one of Canadian, the other of New Zealand manufacture, which had been held in a frozen state (in cold store) for about 7 years were presented to this Institute.

FLAVOUR

As received, both cheese were rather dry, but the Canadian was 'moist' and the New Zealand 'short' in texture. They had acquired a strong 'Cheddar' flavour, and rancidity and tallowiness were also apparent. This strong flavour was not generally approved, especially by the younger members of the staff, though only a few found the cheese altogether inedible.

BACTERIAL FLORA

Very few living bacteria and no moulds were found. Only spore-bearing bacteria and occasional Group D streptococci appeared to have survived the storage conditions. There was doubtful lipolysis on tributyrin agar plates, and the spore-bearing bacteria liquefied gelatine.

CHEMICAL COMPOSITION

Analyses, with special reference to the protein breakdown and volatile acids, were made for comparison with data available for normally ripened Cheddar Cheese and obtained by the same methods of analysis (1). In Table 1 the data are given for the present two samples and three normal mature Cheddar cheese.

Table 1

				Protein breakdown (% of total N)			Volatile acids (ml. N/10-acid/100 g. of dry matter)			
Cheddar cheese sample	Age of cheese	Moisture (g./100 g. cheese)	pH	Non-protein N	Non-protein Sörensen N	NH_3-N	Total	Water fraction	Fat fraction	Water insoluble
Canadian	7 year	24·6	5·54	41·1	13·4	5·1	118·1	74·6	43·5	16·1
New Zealand	7 year	20·2	5·54	34·8	12·6	4·9	134·7	85·6	48·9	27·7
N.I.R.D. IV C_2	224 days	33·7	—	27·9	14·8	3·72	200·6	157·3	43·4	9·95
N.I.R.D. II C_3	205 days	29·2	4·87	24·3	8·8	1·8	110·7	69·4	41·3	12·0
N.I.R.D. V C_{13}	83 days	36·0	5·13	22·1	9·7	2·1	96·9	65·6	31·3	11·9

VOLATILE ACIDS

The volatile acids were determined by an extraction method (1). Since the moisture contents of the cheese were abnormally low the volatile acids were calculated for 100 g. of dry matter, and the figures given by Hiscox, Harrison & Wolf (1), used for comparison, were re-calculated on this basis.

In spite of the advanced age of the two samples the quantities of volatile acids were not unduly high, and it would appear that the conditions of the long storage period were not

such as to induce an exceptionally strong lipolysis of the fat, although the high proportion of water-insoluble higher fatty acids indicates that lipolysis had proceeded further than is usual in normal Cheddar cheese.

PROTEIN BREAKDOWN

The extent of the protein breakdown was measured by the method of Davies (2), involving determinations of the total nitrogen, non-protein nitrogen not precipitable by 4% trichloroacetic acid, non-protein Sörensen nitrogen, i.e. amino-nitrogen in the trichloroacetic acid filtrate, and ammonia nitrogen. In Table 1 the amounts of the various fractions are expressed as percentages of the total nitrogen, and it will be seen that the extent of the protein breakdown was greater than that found in normally ripened Cheddar cheese.

ACIDITY

The greater extent of the protein breakdown, especially the higher percentage of ammonia nitrogen, is reflected in the higher pH of the two samples.

I am indebted to Mr J. Davidson for the data on protein breakdown, acidity and moisture.

REFERENCES

(1) HISCOX, HARRISON & WOLF (1941). *J. Dairy Res.* **12**, 155.
(2) DAVIES, DAVIS, DEARDEN & MATTICK (1934). *J. Dairy Res.* **5**, 144.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

I. REPRODUCTION AND LACTATION

The main purpose of the present review is to give a brief account of the research work which has been published on reproduction and lactation in dairy cattle since the previous review of this series appeared in 1945(1). Earlier papers are cited only where it was found essential, in order to explain the meaning or importance of the more recent work. It is not intended, nor would it be possible in the space available, to trace even briefly the history of any aspect of the subject from its beginning. Throughout the review, however, reference is frequently made to other reviews which have appeared recently and which, since they deal with only one specific aspect of the subject, include more details of earlier work.

REPRODUCTION

One of the major problems causing wastage in dairy herds is sterility. Figures published a few years ago for some counties in England(2) showed that in 48 herds containing 1925 cows, there were 620 disposals in one period of 12 months. Of these disposals 23% were due to sterility. The subject has formed the basis of a series of review articles published recently in the United States. In one of these articles Bartlett(3) discusses infectious diseases as a cause of infertility, and in two others Asdell, who has done so much work on the subject, discusses the treatment of sterility by nutritional methods(4) and with hormones(5). In the same series Gilmore deals with the inheritance of functional causes of reproductive inefficiency(6), and Sykes reviews possible methods by which the problem should now be investigated(7). Sykes rightly points out that while much is known of the effect of genetic, pathological, nutritional and physiological factors on reproductive processes, the applicability of much of this information to problems of infertility as encountered on the farm has not been determined as fully as it ought. He also stresses the need for more general agreement as to what constitutes infertility and of more reliable methods of diagnosing its different forms. In his view too much reliance is placed at present on the number of services per conception and other data of that sort which is not adequate for the purpose. In Britain, Laing(8) has also stressed the absence of reliable information on the incidence of different types of infertility, and points out that much more knowledge of the aetiology of infertility will be required before the diagnosis can always be relied upon and the correct treatment applied. For a valuable clinical analysis of reproductive failure in cattle at the Wellcome Veterinary Research Station the reader should refer to a paper by Hancock(9).

The main pathological causes of sterility and of modern methods of treatment with particular reference to conditions in Holland have been discussed by van den Burg(10), and somewhat similar information for Ayrshire herds in Finland by Korkman(11). Hornaday(12) states that cystic ovaries occurred in 5·8% of the cows in the herds he attended, and mummification of the foetus in 5% of the breeding females. He has observed the occurrence of this condition in about every second conception in cow families for two, three and four generations. In experiments in Germany, Wille(13) found that in sterile cows 2% showed a normal uterine condition, 36% had some form of uterine inflammation, and 62% had various forms of metropathy. After treatment which involved douching the os uteri, all the cows with normal uteri and almost all the cows suffering from metropathy became pregnant; of those which had inflammatory conditions only 53% conceived after treatment. In Canada, Durrell(14) studied the effect of treatment with natural and synthetic hormones on sterility in 39 cows ranging in age from 1½ to 12 years and which had failed to conceive after at least three services. After treatment with pregnant mares' serum nearly 50% of the treated cows conceived. There appeared to be no relation between

infertility and advancing age or high milk production. Folley, Greenbaum & Roy(15) and Greenbaum(16) have studied the response of the goat to the gonadotrophin of pregnant mares' serum. They found that of forty-four anoestrous goats which were given single injections of 1200 i.u., thirty-five came into oestrus and were mated. Ten of the forty-four gave birth to young, and no super-foetation was encountered. The clinical use of luteinizing hormone applied to twelve cows and heifers showing ovarian cysts has been discussed by Hancock(17).

Quinlan and his colleagues in South Africa(18) have published the results of their long-term investigation on sterility in cows which began in 1929, and in which the problem studied included the effect of dry rations, lack of exercise and lack of sunlight on reproduction in beef heifers and cows. It was found that restriction of sunlight and exercise did not appear to have a detrimental effect on the health and growth of the animals or to delay the onset of maturity. Up to the age of 31 months the mean number of dioestrous cycles was 15·5 in the restricted groups compared with 12·2 in the unrestricted group, the average period of dioestrus for all groups being 20 days. Restriction of sunlight and exercise had no significant effect on the length of the period between calvings, the conception rate, the length of gestation or the weight of the calves produced.

The effect of season, of the hours of daylight and of the age of cattle on reproduction and milk production continues to be widely investigated. After extensive work involving large numbers of cows and bulls in eastern Canada and in New York State, Mercier & Salisbury(19, 20) concluded that fertility in different months was closely related to the average length of daylight for each month, there being a lag of 1–2 months before the maximum effect was reached. Temperature changes had no measurable direct effect on fertility, and the response to light varied with the age of the animals. It is suggested that light influences reproductive functions in cattle by influencing the pituitary indirectly through the eye and the optic nerve. Variations in the fertility records of two groups of bulls in different seasons of one year have been compared by Laing(21). One group was used for controlled service (a double service as soon as oestrus was noticed), and the other for free service, the cow being left with the bull throughout oestrus. The fertility of the free-service group appeared to be better than that of the other except from December to March. This difference was correlated with the varying length of the oestral periods at different seasons of the year, and it is suggested that the higher fertility of the free-service group resulted from better timing in relation to ovulation.

The conception rate in the Nebraska University herd has been studied over an 8-year period by Timberger & Davis(22). The average number of services per conception in twenty cow families involving 479 conceptions was 1·72. The conception rate of 2·24 in August was significantly higher than that in other months of the year, when it varied from 1·45 to 1·90. One of the cow families had a significantly higher conception rate (2·92) than the others, and two families had significantly low rates (1·22 and 1·00). In one family of sixteen cows, six were sterile, an indication of inherited sterility. Analyses of 204 breeding records for the daughters of nineteen bulls revealed one with daughters that required 2·25 services per conception, a number which was significantly higher than the average of 1·71 for the 204 cows.

The effect of month of calving on milk production has been the subject of informative publications from Norway(23, 24, 25), Australia(26), United States(27, 28) and Wales(29, 30, 31). Some of the Welsh data was collected from 103 herds, averaging 15·7 cows per herd. In this particular sample 25% of the calvings occurred in autumn between October and December, 27·4% between January and March, 17·9% between April and June and 29·5% between July and September. The percentage of autumn calvers decreased as the size of the herd increased. Only 22% of the cows reached their sixth lactation, and the average number of times a cow calved was 3·1 for all herds, and ranged from 3·4 in the smallest herds to 3·0 in the two largest herds. For 25,000 pedigree and 50,000 non-pedigree cows in recorded herds in England and Wales, the average number of calvings per cow has also been found to be 3·1(32). From an extensive study of seasonal variations in the sale of calves in different parts of Britain from 1943–7, Phillips, Brown & Davies(33) have concluded that climate may have both direct and indirect effects on the development of winter oestrus in cattle. In some of the Norwegian work(25), data collected from 1911 to 1945 showed

that cows which were 20–25 months old at their first calving tended to have a slightly longer productive period than those calving for the first time at 26–40 months old. When cows calved for the first time at under 20 or over 40 months their productive period was considerably reduced. The length of the dry period has been found for Finnish Ayrshire cattle(34) to have only a very small effect on the amount of milk produced in the next lactation. For Bohemian, Moravian and Silesian cows(35) the average weight of the newborn calf tended to decrease when the preceding dry period of the cow was reduced. When in cows under 5 years old the dry period was shortened by 10 days, the weight of the calf was decreased on the average by 0·24 kg. For cows over 5 years old the average decrease was just over 0·45 kg.

The length of the gestation period in different breeds of cattle has been studied again in recent years. In west Virginia(36) it has been found that the average for Aberdeen-Angus cows was 282·5 days and for Herefords 285·2 days, the difference being statistically significant. For three dairy breeds, Jerseys, Ayrshires and Holsteins, the corresponding figures were 277·9, 277·8 and 278·3 and the differences were not statistically significant. There was therefore no evidence that an early-maturing dairy breed like the Jersey has a shorter gestation period than the slower-maturing dairy breeds. The difference of almost 6 days between the beef breeds and the dairy breeds was highly significant. In the two beef breeds the length of the gestation period was not affected by the sex of the calf. In the three dairy breeds the average figure for 637 births giving male calves was 278·4 days, as compared with 277·5 for 623 births giving female calves. In Brazilian bred Friesians(37) 94% of the gestations lasted between 260 and 289 days with an average of 276·2. Male calves predominated in the longer gestations and female calves in the shorter. In Brown Swiss cattle(38) the range was 270–306 days, with an average of 291. This breed appeared therefore to have a longer gestation period than most other breeds. The sex of the calf, however, did not have any significant effect on the length of the period. In Zebu breeds in India(39) the period was found to be 292·5 days, and although the mean for gestations resulting in male calves was 3 days longer than the mean for those resulting in female calves, the difference was not statistically significant.

The early diagnosis of pregnancy which was discussed in the previous review(1) is of great importance for maintaining reproductive efficiency in cattle, but in the past few years little further development appears to have occurred. Casida and his colleagues at Wisconsin(40) have used a method which depends on palpation of the amniotic vesicle, and have compared their results for over 3000 inseminations with herd-book records. When the diagnosis was made between 30 and 60 days after insemination the discrepancy amounted to 15·3%; when made between 60 and 90 days it was 6·1% and when made between 90 and 120 days it was 3·0%. Brigatti(41) has investigated the possibility of using the morphology of the corpus luteum as a possible help in the early diagnosis of pregnancy in the cow, but he found no difference between pregnant and non-pregnant cows in the size, position and conformation of the corpus luteum.

A thorough study of the development of the egg of the cow up to the stage of blastocyst formation has been made by Hamilton & Laing(42). Ova were collected from twenty-eight heifers and eighteen cows which had been artificially inseminated. Cleavage stages are described, the diameters and volumes were calculated for each stage, and the data are compared with those of other mammalian ova. Since transplantation of ova may be a natural development of artificial insemination Rowson & Dowling(43) have studied the problem and have devised an apparatus for the extraction of fertilized eggs from the living cow.

The levels of ovarian hormones required to induce heat and other reactions have been investigated by Asdell and his associates at Cornell(44). In ten ovariectomized heifers the average minimal dose of oestradiol benzoate required to induce heat was 600 rat units for 3 days, but there was a very wide variation in the individual values which ranged from 366 to 800 rat units. One heifer failed to respond to doses of up to 5000 rat units, and another to doses up to 70,000 rat units. The duration of heat was almost always less than 1 day, even with high doses over long periods. In three heifers and a freemartin the minimum average level of stilboestrol which would

induce heat was about 660 rat units or 0·26 mg. daily, the range being 0·13–0·30 mg. The freemartin required the highest dose.

A most valuable study has been made by Laing(45) of the characteristics of the semen of bulls in relation to their fertility. It was found that on the basis of the density of the semen, the total number of spermatozoa ejaculated and the motility and change in pH of the semen on incubation at 37° C., the animals could be divided into a higher fertility group with service rates from slightly more than 1·0 to about 3·5 per conception, and a lower fertility group with service rates above 3·5. Totally sterile animals could also be detected. No relationship was detected between the percentage of abnormal spermatozoa ejaculated and the service rate. Laing(46) has also done extensive work on the survival time of spermatozoa in the genital tract of the cow and its relation to fertility. In a portion of the investigation five cows and twenty-nine heifers were inseminated with a known number of spermatozoa at various times in relation to the end of normal oestrus or oestrus induced by expression of the corpus luteum. Inseminations made earlier than about 16 hr. before the end of heat were not fertile; those made between the end of heat and 16 hr. before it were all fertile; after the end of heat the results were irregular. For an account of other important findings, too numerous to mention in the brief space available here, reference should be made to the original paper.

A synthetic pabulum which may be used for the preservation of bull semen has been described by Phillips & Spitzer(47). In a trial involving semen from nineteen bulls and over 1200 inseminations(48), the fertility was regarded as being up to 23% higher in semen diluted with egg-yolk citrate buffer than with other portions of the same semen diluted with the synthetic pabulum. Many other papers on bovine semen and spermatozoa have been published during the past 5 years, but it will be possible to refer to them only very briefly here. Some deal with the effect of using different dilutions of semen(49, 50, 51), and of different types of diluent(52, 53, 54, 55) on motility and fertility; others discuss the use of sulphanilamide in the diluent at different levels of dilution(56, 57, 58, 59). The metabolic activity of the spermatozoa(60), their damage by oxygen and the reduction of that damage by catalase(61), the hyaluronidase content of bull semen(62, 63, 64, 65) and the freezing-point of bull semen(66) have all been studied in considerable detail. Almquist and his associates at Pennsylvania(67) have found no apparent relationship between the average plate count of semen and the general fertility of bulls, nor did the age of the bulls have any important effect on the plate count. They have also studied the incidence of specific types of bacteria in bull semen(68). Branton, Bratton & Salisbury(69) found that when fertility was judged by the characteristics of the semen, milk protein fed as a dietary supplement did not result in greater fertility than soya-bean and maize proteins.

A very detailed study of bull spermatozoa has been made in Delft with the aid of the electron microscope at magnifications up to × 50,000(70, 71, 72). The morphology of normal and abnormal spermatozoa is described. The position of the chromosomes in the sperm head is demonstrated, and the main abnormalities in spermatozoa from sterile bulls are classified and illustrated. Factors which cause disturbance of spermatogenesis are discussed. In recent work by Gresson & Zlotnik(73) the position, appearance and behaviour of the various cytoplasmic components during oogenesis and spermatogenesis in the bovine have been described and discussed.

HORMONES

In the previous review published in 1945(1), a documented account was given of theories on the hormonal control of mammary development and lactation. Two years later the subject was discussed in greater detail by Folley(74, 75). It will be possible here to mention these theories only very briefly and then to refer to some of the more important publications which have appeared since the 1945 review was written.

The development of the mammary gland. The theory of mammary development which received most prominence a few years ago was that advanced by Turner and his associates at Missouri. This theory postulated that under the influence of oestrogens the anterior pituitary secretes

a hormone called Mammogen I, which stimulates growth of the duct system of the gland, and that under the influence of progesterone the anterior pituitary secretes another hormone, Mammogen II, which is responsible for the growth of the lobule-alveolar tissue. Little further evidence has accumulated in recent years in support of this theory, but Reece & Leatham(76) appeared to confirm it in part when they found that in experiments with castrated hypophysectomized rats, oestrogen alone did not stimulate mammary duct growth, whereas oestrogen with pituitary lactogenic preparations caused duct growth similar to that obtained by injecting oestrogens into intact rats. Trentin & Turner(77) attempted to separate the duct and alveolar growth-promoting activities by fractionating anterior pituitary preparations, but they did not succeed.

Other workers, for example, Cowie & Folley(78, 79), have found that crude anterior pituitary extracts will cause mammary growth in gonadectomized animals, but it is still unknown whether these affects are caused by new and distinct hormones such as Mammogens I and II, or whether the active principles are simply one or other of the hormones already known to be secreted by the anterior pituitary. The evidence against the Mammogen theory has been discussed in earlier reviews(1, 74), where it is pointed out that various workers have obtained at least limited mammary growth in completely hypophysectomized animals with oestrogen treatment alone, and that frequently when one rudimentary teat is treated with oestrogen that particular teat develops while the neighbouring teats show little or no change, a fact which does not support the view that the oestrogen acts through the pituitary, for if it did so act, both the treated and untreated glands would be expected to develop together. And certainly in some species oestrogen alone without any progesterone treatment can result in the development of fully developed mammary glands capable of secreting large volumes of milk. This has been clearly demonstrated by many workers, including Folley & Malpress(80) for a freemartin, Folley, Malpress & Young(81) with goats and bovines, and by Day & Hammond(82) and Spriggs(83) for bovines. Some years ago, on the basis of histological studies, Mixner & Turner(84) suggested that the mammary tissue developed in goats by oestrogens alone was not normal, and that it became normal only when progesterone was also given, but as Malpress(85) points out Folley and his colleagues at Reading have also investigated this problem and tend to the view that progesterone does not lead to alveolar tissue superior to that obtained by oestrogen alone.

More than ten years ago Van Heuverswyn, Folley & Gardner(86) found that desoxycorticosterone promoted mammary duct growth in immature male mice. Cowie & Folley(78, 79) have now endeavoured to determine more precisely the part which the adrenal cortex plays in mammary development in the rat, and for this work they devised a semi-quantitative method of studying changes in the structure of the gland. They found that an ox anterior-pituitary extract showed mammogenic activity both in the presence and absence of the adrenals. Although there was some evidence that mammary development was sometimes upset by adrenalectomy, the mammogenic action of the pituitary extract was not primarily due to the secretion of adrenal cortex hormones. In a later publication on relative growth of the mammary gland, Cowie(87) found that adrenalectomy did not affect the relative growth rate of the gland in intact female rats, and that in the male it appeared to cause a slight increase of doubtful significance. By the same technique the essential part played by the ovaries was clearly demonstrated. It seems, therefore, that at any rate in rats the adrenal glands do not make any important contribution to mammary development. This view, however, is not held by Trentin & Turner(88), who found that the alveolar development produced by hormone treatment in castrated rats was greatly reduced after adrenalectomy, although some duct development still occurred.

In 1944 Petersen and his associates(89) found that in the thyroidectomized bovine, no mammary development was obtained by oestrogen treatment unless thyroprotein treatment was first applied, and in 1947 Mixner(90) concluded that thiouracil and thyroidectomy both decreased the responsiveness of the mammary glands of mice to mammary growth-promoting hormones. In rats, on the other hand, Leonard & Reece(91) and Smithcors & Leonard(92) had previously found that although thyroidectomy inhibited duct growth it favoured the stimulation of lobule-alveolar development. Thiouracil, however, did not have the same results(93). The effect of thyroidectomy on mammary

development is therefore not yet very clear, and as Folley(74) rightly points out, it is important when attempting to interpret the results obtained in experiments of this type to remember that it is extremely difficult and perhaps almost impossible to remove the thyroid completely at any rate in the rat.

LACTOGENIC HORMONES

The anterior pituitary. The outstanding work which has been published under this heading since the previous review was written(1) is that by Folley, Young and their associates(94, 95, 96). The first experiments of this series(94) involved eighty-five cows in the declining stage of lactation, and included Ayrshires, Dairy Shorthorns, Friesians, and cows of the South Devon breed. Preparations made from whole ox pituitary tissue and also from anterior lobes were injected subcutaneously before or during the afternoon milking. When a dose of crude alkaline extract of fresh ox anterior-pituitary tissue equivalent to 2·5 g. of fresh gland was given every second day for 3 weeks, there was an average increase in milk production of 20% above the yield which would have been expected in the absence of treatment. Even during the 2 weeks following the treatment there was a 15% increase. Similar responses were also obtained with extracts of the whole pituitary tissue and with an extract of anterior lobe tissue which had been desiccated with acetone. When ethanol was the desiccating agent, however, the extracts were almost inactive. All the breeds reacted to the treatment, and repeated injections of extracts from whole pituitary tissue did not interfere with normal pregnancy and parturition. On this experimental scale the profit to the farmer from the extra milk produced in the 3-week period of treatment (15*s.*) more than covered the cost of the treatment itself (11*s.*), but a difficulty in applying this treatment commercially would be the scarcity of pituitary tissue.

In the second paper of the series Fawns, Folley & Young(95) found that similar treatment administered to cows at the peak of lactation did not result in an increase in milk yield. It would appear, therefore, that for a few weeks after parturition the factor limiting the output of milk is not the rate of secretion of the galactopoietic hormones of the anterior pituitary. In cows calving normally this becomes a limiting factor only after the decline in lactation has set in, namely, some 7–10 weeks after parturition. Two cows which aborted, however, responded to the treatment within 3 weeks of parturition.

In the third paper, Folley, Malpress & Young(96) describe experiments designed to compare the galactopoietic activity of extracts of pituitary glands from different species. When single injections were given to cows in declining lactation, horse pituitary extract was more active in stimulating milk production than ox pituitary extract, whereas extracts from the pituitaries of sheep and pigs tended actually to depress the milk yield. Since horse pituitary is known to be relatively deficient and sheep pituitary to be relatively rich in prolactin, the galactopoietic effect was obviously dependent on factors other than prolactin. The authors point out that the effect might possibly be related to the content of follicle-stimulating hormone in the glands of the different species; this might stimulate oestrogen secretion by the ovaries of the treated animals which might in turn make their pituitaries secrete more actively. The authors suggest that the depressing effect of the sheep and pig extracts might have been due to the rapid formation of antihormones. In 1949 Cotes, Crichton, Folley & Young(97) showed that a purified preparation of the anterior pituitary growth hormone was highly galactopoietic. In fact, it was calculated that in short-term experiments the whole of the galactopoietic activity of an anterior pituitary extract might reasonably be attributed to the growth hormone. The authors point out that treatment with pituitary growth hormone may induce growth, the secretion of extra milk or diabetes depending on the age, species and condition of the treated animal. In 1947 the work with cows was reviewed by Young(98), and experiments on the use of anterior pituitary and other hormone preparations in women by Robinson(99, 100).

In experiments by Meites & Turner(101) thiouracil in the diet of rats reduced the lactogenic hormone content of the pituitary as estimated by the response of the pigeon-crop gland. From that and other findings the authors concluded that the lactogenic hormone originates in the acido-

phil cells of the anterior pituitary. The same authors(102) have described the induction of lactation during pregnancy in rabbits by injecting prolactin. Liu & Turner(103) have made the interesting suggestion that there may be a relationship between the amount of lactogenic hormone excreted in the faeces of the cow and its milk-producing ability. They found that the average yield of lactogenic hormone from 1 kg. dry faeces was 0·29 to 1·25 i.u. for high milk producers, 0·23 to 0·30 i.u. for low yielders and 0·17 to 0·19 i.u for bulls.

The investigations by Li and his colleagues on the properties of the lactogenic hormone have been continued and extended in recent years(104, 105). For a description of the preparation and chemistry of the anterior pituitary hormones reference should be made to a review by White(106).

Adrenal cortex. In 1943 Nelson, Gaunt & Schweizer(107) described experiments which suggested that when prolactin and cortical steroids initiate lactation in the absence of the pituitary, the steroids concerned are those which stimulate gluconeogenesis (11-oxygenated steroids) rather than those connected with the metabolism of salts (11-deoxy steroids). The subject has recently been studied with rats by Cowie & Folley(108, 109), who found that adrenalectomy on the fourth day after parturition led to inhibition of lactation which was usually only partial. Daily administration of 3 mg. deoxycorticosterone acetate gave a 60–100% restoration of lactation, the response varying directly with the degree of lactational inhibition caused by adrenalectomy. In one experiment where removal of the adrenals caused complete inhibition, the dose just mentioned completely restored lactation to the control level. The deoxycorticosterone derivative was found to be more effective in these particular experiments than 11-dehydrocorticosterone or an adrenal cortex extract, and with the latter two treatments a high-protein diet reduced the small response which they caused but did not greatly affect the response brought about by the deoxy-derivative. It would appear therefore that in these experiments by Cowie & Folley the absence of the 11-oxygenated steroids which are concerned with protein and carbohydrate metabolism was not the chief cause of lactational inhibition resulting from adrenalectomy. In later work Folley & Greenbaum(110) found that the increase in weight which they observed in the mammary glands of adrenalectomized rats treated with deoxycorticosterone was not due to cellular hyperplasia but to a greater water content in the glands involving increased cellular hydration, oedema or both. The same authors(111, 112) have studied the effect of adrenalectomy and treatment with adrenal cortex hormones on the arginase and phosphatase levels of livers, kidneys and mammary glands of lactating rats on normal and on high-protein diets. They found that adrenalectomy reduced the amount of arginase to almost one-seventh its normal value in the mammary gland, to about a half in the kidney and to about a third in the liver. Although the hormone treatment did not restore the arginase content of the mammary gland, the results following adrenalectomy were believed to suggest that the adrenal cortex influences lactation directly through the arginase of the mammary gland and indirectly through the liver arginase. In connexion with their extensive work on hormones and lactation Cowie & Folley(113) have described their method of measuring lactational performance in the rat by using what they term the 'litter-growth index', which is defined as the mean daily gain in weight per litter over the 5-day period from the 6th day to the 11th day. Partial inhibition of lactation is assessed by expressing the litter-growth index of treated rats as a percentage of that of the controls.

So far there seems to be no clear confirmation for the suggestion made some years ago that a specific lactation hormone is secreted by the adrenal cortex.

Oestrogens. The object of most of the work which has been done with oestrogens in the past few years has been to study what is sometimes termed the artificial induction of lactation. This subject was dealt with in the previous review(1), and has since been discussed in greater detail by Hammond(114) and Malpress(115). It would be fruitless to attempt to review here the numerous papers on this subject which have been published in the last few years in many parts of the world. However, it may be useful to the reader if some of the more important publications are cited, even although they are not discussed. Experiments on the artificial induction of lactation, mainly with synthetic oestrogens, have been made with varying degrees of success, using heifers and cows(116, 117, 118, 119, 120, 121, 122, 123, 124), goats(116, 125), sheep(126) and mares(127).

One of the most interesting discoveries of recent years which has a bearing on the effect of oestrogens on milk production is that of Bartlett, Folley, Rowland, Curnow & Simpson(128), who have shown the presence of oestrogens in spring grass. It will be of great importance if further work shows that the galactopoietic effects of fresh young pasture is due not only to its richness in digestible nutrients but also to its possession of hormone activity.

The thyroid. Nearly 16 years have elapsed since Graham(129, 130) and Folley & White(131) showed conclusively that the administration of thyroxine to cows in the declining phase of lactation can raise the yield of milk and milk fat. These observations, coupled with the discovery of Ludwig & Mutzenbacher(132) that iodinated casein possesses thyroxine activity, have led in most of the principal countries of the world to an intensive study of the effect of thyroxine and iodinated protein on the production and composition of cows' milk. It will not be possible here to do more than mention very briefly some of the more important papers which have been published on the subject in the past five years.

In 1945 descriptions of the preparation and biological effects of iodinated proteins were published in Britain by Pitt Rivers & Randall(133) and Deanesly & Parkes(134). In the same year Blaxter(135, 136) gave an account of the numerous carefully conducted experiments which he began in 1941 and which were designed to determine whether milk production could be stimulated by iodinated proteins under normal farm conditions without harming the health of the cow. A year later(137) he published further extensive experiments which were carried out on farms in England and Wales and involved more than a thousand cows and six different breeds. Blaxter's conclusions from this and from a general review of the literature are to be found in an excellent survey of the subject published by Blaxter, Reineke, Crampton & Petersen(138), which is referred to more fully on p. 347.

Other publications which have appeared in recent years on the general effects of feeding iodinated proteins to cows include those of Van Landingham and his associates(139, 140), Seath, Branton & Groth(141), Reece(142, 143, 144, 145), Booth, Elvehjem & Hart(146), Allen, Dow, Logan & MacKenzie(147) and McQuillan, Trikojus, Campbell & Turner(148). Owing to the fact that in much of the earlier work when 10–20 g. iodinated protein were fed per day, losses in body weight occurred and were accompanied by increased heart and respiration rates, Swanson & Knodt(149) have recently studied the effect of feeding lower levels of iodinated protein to lactating cows daily for 1 year. Dosage levels of 0·625, 1·25 and 5·0 g. were tested with intakes of total digestible nutrients equivalent to 125 and 110% of Morrison's standards. Although in some of the experiments milk production appeared to increase slightly, there were no statistically significant changes in milk yield, body weight or in heart and respiration rates. Also very recently Thomas, Moore & Sykes(150) published the first results of a long-term experiment designed to determine the effects of feeding iodinated protein for several years and throughout several lactations on health and reproduction in cattle. The results are given for five Holsteins and six Jerseys receiving the active protein for about 300 days beginning at the 50th day after parturition. The iodinated protein was fed at levels of 0·6, 1·0 and 1·5 g./100 lb. live weight. It was found that in treated cows loss in body weight could be prevented, and increased milk production sustained throughout lactation by feeding at a level of 25% above the recognized Morrison standard. Heart rate, like milk production, was related to the intake of total digestible nutrients, stage of lactation and intake of iodinated protein, and the gross efficiency of milk production appeared to remain unchanged. It is important to note that Swanson & Knodt(149) wisely point out in the paper already cited that poor producing cows respond less to iodinated protein treatment than high producers, and that there is therefore little point in attempting to make a poor cow into a good cow by this means!

The energy metabolism of lactating cows treated with iodinated casein has been studied in Denmark by Thorbeck, Hansen & Moustgaard(151), and the nitrogen, calcium and phosphorus metabolism of cows treated with thyroxine has been investigated in Britain by Owen(153). In the Danish experiments 25 g. iodinated casein were given daily for 90 days to three Red Danish cows. The resulting increases in milk production varied up to 54%, and the weights of the cows

decreased by 10–17%. During the treatment heat production increased by about 30%, and for the increased milk production during treatment the increase in calorie intake was about three times normal. The heart rate was increased by 40 or 50%, but otherwise no abnormality in heart function could be detected by electocardiography. In experiments involving eight Ayrshire cows, Owen (152, 153) found that the increase in milk production caused by thyroxine was accompanied by loss in body weight and negative nitrogen balances, but that the negative nitrogen balances could readily be inhibited by increasing the food intake. The calcium balances were negative for both the treated and the control cows, and there was no increase in the urinary excretion of calcium during treatment, but in two of the treated cows increased faecal excretion of calcium occurred. Phosphorus balances determined for two of the cows were positive throughout the experiment, and thyroxine treatment tended to increase phosphorus retention. The calcium content of the milk was not affected by the treatment, but the phosphorus content appeared to increase slightly.

A detailed study of the effect of iodinated casein feeding on the metabolism of sheep has been made by Blaxter (154, 155, 156). In the third paper of the series (156) he concluded that the effect of iodinated casein on basal metabolism in sheep is linearly related to the dosage expressed in mg./kg. $W^{0.73}$. From this he calculated that a cow receiving 20 g. iodinated casein per day might be expected to show an increase of some 30% in basal metabolism. Considering that Blaxter's estimate was obtained from data on sheep and that samples of iodinated casein can vary considerably in potency, the estimate agrees well with the increase of 30% obtained in cows by the Danish workers (151) for doses of 25 g./day.

The effect of iodinated protein feeding or thyroxine treatment on the level of various constituents in cows' milk has been discussed by many of the workers in this field. Marked increases in the fat percentage of the milk are usual, but do not always occur, and at least in one set of experiments where prolonged treatment was given (150), the increase appeared to occur for a period of only 30–40 days. The effect on the principal non-fatty constituents seems to be relatively slight and variable (139, 140, 146, 157); there is some evidence that the casein content decreases slightly (146, 157), while the globulin and albumin fraction increases (157). Kemmerer, Bolomey, Vavich & Davies (158) found that iodinated protein feeding had no effect on the carotene and vitamin A contents of milk, but that it resulted in an increase in the concentration of nicotinic acid and a decrease in the concentration of riboflavin. The lack of effect on carotene content was also observed by Hibbs & Krauss (159), but these workers found that decreases in the vitamin A content of both blood plasma and milk sometimes occurred. Van Landingham and his associates (160) observed that when 15 g. iodinated casein were fed per day, the ascorbic acid content of the milk decreased by about 30%, but Hibbs & Krauss (159), on the other hand, found treatment of this type to have no effect on the level of ascorbic acid, though they found a decrease in the aneurin content of the milk. The decrease which is sometimes observed in ascorbic acid content is now believed to be caused by the ingestion of relatively large amounts of iodine in the iodinated protein (138), and certainly when thyroxine itself is given an actual increase in ascorbic acid content appears to occur (161).

If iodinated protein or thyroxine treatment were to be used in practice, it would be essential to ensure that thyroxine did not pass through the mammary epithelium in significant amounts. Robertson (161) reviewed the literature on this subject in 1945, and also described experiments in which a study was made of the basal metabolic rate, pulse rate, and blood pressure in twenty-seven healthy women who drank each day for a month a quart of milk from cows which were fed iodinated protein. No adverse effects were observed; in fact a significant increase in body weight occurred. In experiments by Bruger & Silberbush (162) twelve children, aged 9–14, were fed each day for 116 days 720 ml. milk from cows receiving 15 g. iodinated protein per day. A control group of nine children were given milk known to be normal. No significant differences were found between the two groups in gain in height and weight, in basal metabolic rate or in pulse rate and respiratory quotient. Hurst & Turner (163) have recently demonstrated that the mammary epithelium of the mouse is impermeable to thyroxine.

For a most informative survey of the whole question of iodinated protein feeding to cows the reader should refer to the excellent review by Blaxter and his associates(138) which has already been cited. The principal conclusions arrived at in that article are enumerated below.

(1) Within a limited range the percentage increase in milk yield is roughly proportional to the dosage.

(2) An increase in fat percentage in the milk usually appears with lower doses than an increase in milk yield.

(3) Apart from an increase in fat percentage and a small variable decrease in milk nitrogen, no other very important alterations in milk composition have so far been established.

(4) Thyroxine is not transmitted to the milk, so that the milk from treated cows should have no harmful effects on the consumer.

(5) If sufficient extra nutrients are supplied to meet the requirements of the extra metabolism and the extra yield of milk during treatment, losses in body weight can be avoided.

(6) Iodinated protein might be used profitably during periods of milk scarcity for periods of about 2 months, or possibly for longer periods where the conditions were favourable, but care must be taken not to exceed a dose which can readily be tolerated by the lactating animals.

(7) More information will be required on the long-term effects of treatment before it would be wise to adopt it in pure-bred breeding herds.

Since these conclusions were published Thomas and his colleagues(150), in a very recent publication, have emphasized the truth of the last of these conclusions when they state that 'since all the effects of feeding thyroprotein to cows have not yet been determined, there is no sound basis on which one can make recommendations to the practical dairyman concerning the advisability of feeding thyroprotein'.

Other publications which may be cited include a brief popular review of the subject by Moore & Sykes(164) and an illustrated bulletin by Dyrendahl(165). Dyrendahl's publication includes a valuable review, and a detailed description of the extensive and thorough experiments he has carried out at Stockholm on the effect of feeding iodinated protein for long periods to young Swedish polled cattle of both sexes, and also to pigs and rats. The many factors studied include rectal and skin temperatures, heart and respiratory rates, and the blood levels of calcium, phosphorus, cholesterol, vitamin A, carotene and vitamin C. Body weights and measurements were also recorded. The treated cattle became longer and taller but narrower than the controls, and in spite of their poorer appearance they were somewhat heavier than the controls. Only young growing animals were used, so that the effect on lactation was not included in the investigation.

The stimulation of lactation in humans by feeding dried thyroid gland has been investigated recently by Robinson(166).

Modern methods of synthesizing racemic(167) and L-thyroxine(168) suggest that the hormone itself will soon be as readily available as iodinated protein. Bailey, Bartlett & Folley(169) have therefore investigated the effect on milk production of feeding L-thyroxine to cows at four different dose-levels (25, 50, 100 and 150 mg./day). The extra amount of milk obtained increased up to the 100 mg. dose-level, but at the 150 mg. level there was no further increase. It was calculated that when L-thyroxine is given by mouth, about 16 times as much is required as when it is injected subcutaneously. The authors point out that by feeding L-thyroxine instead of iodinated casein there would be no need for a bio-assay of the material, the total iodine fed would be much less, and there would be no difficulty about unpalatability which is sometimes a problem with the protein preparations.

The ejection of milk. The theories of Ely & Petersen(170) regarding the ejection of milk have received wide publicity and are now well known. These workers suggested some years ago that many sources of afferent stimuli, such as handling the teats, the rattling of milk buckets and particularly the washing of the uddders with warm water, stimulate the posterior pituitary through the central nervous system to secrete a hormone (oxytocin), which increases the intra-

glandular pressure and squeezes the milk from the alveoli and smaller ducts. Afferent stimuli of a different nature, such as fright, cause the secretion of adrenalin, which has the opposite effect and prevents the ejection of milk. On this theory, to get the highest yield at a milking and to get reasonably rapid milking, it would be advisable to milk the animals while the effect of the oxytocin was at its height. Various attempts have been made to determine the ideal time which should be allowed to elapse between washing the udder with warm water and applying the teat cups. In Pennsylvania, Knodt, Reid, Williams & Kesler(171, 172) varied this time interval up to 20 min., but concluded that there was no significant effect on milk production. They stressed the importance of attaching the teat cups 1–2 min. after the udder is washed, not because it results in higher milk yields but because it tends towards the production of cleaner milk. In experiments with eight Shorthorn cows and one Guernsey by Dodd & Foot(173) at Reading, washing the udders with hot water (115–120° F.) immediately before milking had no pronounced effect on yield and quality and no clear-cut effect on the incidence of mastitis. They point out that when Miller & Petersen(174) obtained an increased milk yield by washing the udders with hot water immediately before milking as compared with 20 min. before milking, the 20 min. method was only carried out over a 1-week period, and the difference in yield might have been due simply to the *change* in washing technique. In further extensive work Dodd & Foot(175) found that the temperature of the udder washing water had no measurable effect on the yield of milk or speed of milking, and in an experiment involving ten cows and lasting 6 weeks it was found that leaving the teat cups on the teats at each milking for twice as long as was necessary did not reduce the yield and quality of the milk or the rate of milking. Continuing these important investigations Dodd, Foot & Henriques(176) compared two established routines, one in which udder washing, fore-milking and concentrate feeding were done less than 1 min. before milking, and one in which they were done more than 20 min. before milking. There seemed to be little difference in the results obtained by the two routines, but the authors concluded that milking immediately after preparing the udder was the more efficient procedure.

At Wisconsin, Ward & Smith(177) have made further experiments in which the washing technique was applied to five cows and the teat cups attached after varying intervals. During the experimental periods one-half of the udder was used as a control and milked 2 min. after washing, whereas the other half was milked 4, 8, 12, 16 or 20 min. after washing. Each half of the udder served alternately as control. Within one experimental period, each of five treatments was assigned to a different cow, and in the course of five experimental periods each cow was subjected to every treatment. The authors found that when the cows were milked 12, 16 and 20 min. after washing, there was a significant decrease in milk yield as compared with that obtained after 2 min. With intervals of 4 and 8 min. the differences were not significant. They suggest that Knodt *et al.*(171) obtained no difference up to 20 min. because their cows possibly became unconditioned to the stimulation of washing over the periods of 35, 70 and 90 days used by Knodt. On the other hand, Knodt and his colleagues(172) suggest that little confidence can be placed in results obtained from experiments such as those of Ward & Smith in which one-half of an udder is treated differently from the other half. Some of the factors influencing the rate of milk flow during milking have been studied also by Korkman(178) in Sweden. He found that the highest initial rate of flow was obtained when the udders were massaged with a towel dipped in water at 130° F. immediately before use and the teat cups applied 1 min. later, but the total yield was not affected.

It would appear from the results so far available that the routine of applying the teat cups 1–2 min. after preparing the udder is generally desirable on grounds of convenience, simplicity of working and clean milk production, but not because it results in a significant increase in yield. Whatever doubt may still exist on the effect of the time interval between washing the udders and the actual milking, the fact that oxytocin is one of the chief factors involved in the 'let down' of milk is clearly beyond dispute. Recent confirmatory work by Smith(179) showed that when cows were milked normally every 2 hr. there were great differences in yield from one milking to another. When, however, 10 i.u. of oxytocin were injected just before milking, the variability

in yield was very greatly reduced. This indicated that the cows were incapable of responding at such frequent intervals to the normal stimulus of milking. Korkman (178) concluded that the cow's reaction to different milking routines depends greatly on the individual animal, and a few years ago Petersen & Gilmore (180) reported briefly an example of inherited sensitivity to the milking environment.

From observations involving one lactating woman Newton & Newton (181) have confirmed that the oxytocin theory for milk ejection in other animals applies also in humans. Milk yields of 168 g. were obtained in normal suckling, whereas when suckling was accompanied by distracting the mother and injecting normal saline the yields were only about 99 g. When the same distractions were accompanied by injections of posterior pituitary hormone, the yield was 153 g.

The process of suckling in calves has been studied further by Smith & Petersen (182). When calves suckled nipples attached to closed tubes the negative pressure developed varied from 12 to 18 in. Hg. When the calves suckled cows the corresponding range was 9–16 in. No milk was obtained when a tube in the mouth of the calf prevented the development of a negative pressure. It was concluded that the calf cannot get milk purely by the compressing action of the jaws. The same authors (183) have studied the effect of varying the negative pressure and pulsation ratio on the speed of milking, but obviously further work is required before the ideal conditions are known. Whittleston (184) has also made a study of this problem. Suckling and the physiology of milking has been ably discussed in a recent review on the nervous system and lactation by Folley (185).

BIOCHEMICAL ASPECTS

An outstanding review dealing with biochemical aspects of lactation has been published during the past year by Folley (186), who has himself made so many valuable and original contributions to existing knowledge on the subject. Brief mention of some of his main conclusions is given here with appropriate references to original work published in the last few years. Folley first discusses problems of technique, and points out that the arterio-venous blood sampling methods which were used so much some years ago to study the question of milk precursors are not so reliable for quantitative work as was once thought, though the use of Nembutal for anaesthetizing the cows has been shown by Shaw (187) to minimize one obvious source of error. The perfusion of the excised udder and the incubation of mammary tissue slices are techniques which have given valuable information in recent years, and in the future the use of tracer isotopes may be expected to elucidate some of the problems now awaiting solution. Already in the past year ^{14}C and deuterium have been used in studying milk-fat formation (188, 189) and ^{32}P in investigating the phosphorus metabolism of the mammary gland (190).

The process by which lactose is formed in the mammary gland still remains to be elucidated. Folley (186) has discussed the subject very fully in his review and puts forward a possible theory based on the assumption that the processes involved in the enzymic conversion of galactose-1-phosphate to glucose-6-phosphate, claimed to have been effected by Trucco, Caputto, Leloir & Mittelman (191) are reversible. According to this theory glucose would pass via glucose-6-phosphate to glucose-1-phosphate, which could be converted to galactose-1-phosphate. This compound might then combine with glucose or glucose phosphate to give a lactose phosphate which would be removed irreversibly from the reaction. The fact that lactose can be formed from glucose in the mammary tissue of the guinea-pig has been shown by Grant (192, 193) and has been confirmed recently by Malpress & Morrison (194). Knodt & Petersen carried out similar experiments with bovine mammary tissue, and showed (195) that when the glycogen content of excised mammary glands was low, it could readily be raised to a normal value by perfusion with large amounts of glucose, and that on incubating the perfused tissue glycogen appeared to be converted to lactose. Lactose also appeared to be formed in tissue slices when glucose, glucose + lactic acid or maltose was the substrate. Experiments (196) in which udders were perfused with bloods of normal and

high blood-sugar content with and without insulin suggested that in the bovine gland lactose may not be synthesized direct from blood sugar but that glycogen may be an intermediate product. The lower lactose yields when insulin was present were taken as evidence that insulin blocks the conversion of glycogen to lactose. These findings, however, have not yet been confirmed. In fact, Malpress & Morrison (194), who readily demonstrated the formation of lactose from glucose in guinea-pig tissue slices, were quite unable to obtain lactose formation from glucose, maltose or glycogen when they used bovine mammary tissue. In describing their experiments Malpress & Morrison make a critical assessment of earlier claims regarding lactose synthesis direct from glucose, and conclude that it has not yet been demonstrated conclusively for bovine tissue *in vitro*.

Another possible method by which at least part of the lactose might arise is suggested by the recent results of Folley & French (197) and of Friedmann (198). There are several links in the chain of evidence. First, as long ago as 1913 Hammond (199) suggested that glycoprotein might be a precursor of lactose and casein; later in 1938, Smith, Howat & Ray (200) drew attention to what they believed to be glycoprotein in abnormal milk which was secreted by cows during inanition and in which the lactose content was abnormally low, and suggested that it might be an intermediate in the secretion of milk solids; later still in 1941 Reineke, Williamson & Turner (201) demonstrated that glycoprotein was removed from blood as it traversed the udder of the lactating goat. More recently, Folley & French (197) studied the respiration of rat mammary slices using some nineteen different carbohydrates or carbohydrate derivatives as substrate and found that only mannose and glucose caused enhanced activity; the others, such as fructose, galactose and lactose, were all inert. Lastly, Friedmann (198) has found that in the glycoprotein of mares' blood serum mannose and galactose are present in equimolecular amounts. These various observations taken together suggest the possibility that at least some of the lactose in milk may well arise from the mannose and galactose of serum glycoproteins.

In the series of experiments by Knodt & Petersen (195, 196, 202), some of which have just been cited, these authors have continued their studies of the carbohydrate metabolism of the mammary gland, using the excised gland and the perfusion technique of Petersen, Shaw & Visscher (203), and also by incubating mammary gland slices. They found that lactic acid was formed in the presence of glucose, maltose, glycogen, pyruvic acid or citric acid, but that pyruvic acid was not determinable either before or after incubation when glucose, lactic acid, citric acid, maltose or glycogen was present. Degradation of both lactic and pyruvic acids appeared to occur only when they were present in large amounts. The fact that lactic acid is not normally utilized by the mammary gland was confirmed by Shaw (187), in an extension of his well-known work on milk precursors. He also concluded that blood pyruvic acid is of little significance in the metabolism of the lactating mammary gland. Knodt & Petersen's experiments (202) on citric acid showed that this acid could be formed from substances already in the tissues and also when glucose, lactic acid, pyruvic acid, maltose or glycogen was added in suitable quantity. Degradation of citric acid was shown to occur when it was present in large amounts. In the same work the utilization of β-hydroxybutyric acid by the mammary gland was confirmed, but it did not appear to give rise to citric acid.

Folley & French (197, 204) have investigated the respiration of lactating mammary gland slices of various species in the presence of carbohydrates, and also of the rat during pregnancy, lactation and involution of the glands. In rat mammary tissue there was an appreciable uptake of oxygen in the absence of substrate indicating oxidation of fat and perhaps protein, but in the presence of glucose and mannose uptake of oxygen was much increased. The activity of tissue from different species, measured by oxygen uptake, was inversely correlated with the body size of the animals studied. It was greatest in mouse tissue and least in cow tissue. In the smaller animals (mouse, rat, guinea-pig and rabbit) the R.Q. of the mammary tissue in the presence of glucose was considerably greater than unity, but in the goat it was 0·69–0·95 and in the cow 0·64–0·93. This would suggest that fat synthesis from glucose readily occurs in the mammary gland of non-ruminants, but that it may not readily occur in that of the cow and goat. As Folley & French

point out the high R.Q. reported in earlier years by other workers for the bovine mammary gland *in vivo* could well be caused by the synthesis of fat from acetate, which has now been shown to occur in ruminant mammary slices. Folley & French (204) found that addition of glucose increased the respiration of mammary gland tissue from the rat during lactation but not during pregnancy. In the absence of substrate the R.Q. was about 0·62 during pregnancy, about 0·75 in lactation and returned to 0·64 at weaning. In the presence of glucose the corresponding figures were 0·83, about 1·6 and 0·76. Again, the likelihood of fat synthesis from glucose in the actively lactating gland of the rat was confirmed.

Much work has been done recently on milk fat and its probable precursors. The most outstanding advances have been made by Folley & French (205, 206, 207, 208) and by Popják, Folley & French (209, 210). These workers have shown by experiments with mammary tissue slices that when glucose is the substrate the R.Q. for the lactating mammary tissue of the rat is greater than unity, whereas for the mammary tissue of cows and goats with glucose as substrate it is less than unity. They have shown also that with 0·02 M-acetate as substrate, little if any of the acetate is utilized by the mammary tissue of the rat, mouse, rabbit, and guinea-pig. Mammary tissue slices of cows and goats, on the other hand, were found to utilize acetate very readily and when they were using it, the R.Q. was greater than unity (205, 206). In later experiments (207) it was shown that mammary gland slices of the rabbit and rat could also utilize significant amounts of acetate provided glucose was also present, and the effect of glucose was shown to be further enhanced by insulin. These experiments, taken as a whole, clearly indicated that at least a portion of the milk fat of ruminants is almost certainly formed from acetate, and that since glucose is always available to the intact mammary gland, acetate may also be one of the precursors of milk fat even in non-ruminants (208). To obtain further information on these points experiments were made with labelled acetate by Popják & Beeckmans (188) and Popják, Folley & French (209). They found that when $CH_3C^{14}OONa$ was injected into pregnant rabbits the glycerides of the mammary glands contained a high concentration of C^{14}, and that the volatile acids contained 7–18 times more isotope than the non-volatile acids. In three of five experiments the butyric and caproic acid fractions had the highest C^{14} content. In the most recent work (210) the labelled acetate was injected intravenously into a lactating goat, and again it was shown that the fatty acids of the milk contained labelled carbon, the lower fatty acids being particularly rich in it.

The arterio-venous sampling technique discussed in earlier reviews (1, 211) indicated that at least a portion of the fat in cows' milk almost certainly comes directly from the triglycerides of the blood, and it is now clear from this excellent work of Popják, Folley & French that another portion is formed from acetate, and that the lower fatty acids in particular are derived in this way and not by oxidation of long-chain fatty acids as was suggested some years ago (1). Since it has now been well established that in ruminants acetate is formed in relatively large amounts in the paunch and absorbed into the blood (212), it is probable that the large amounts of acetate available to the mammary gland of ruminants accounts for the relatively high proportion of lower fatty acids in their milk fat compared with the much lower proportion in the milk fat of other animals.

Within the last few years Mann & Shaw (213) have studied the effect of continuous intravenous feeding of various substances upon the secretion of milk fat in cows. They concluded from Reichert-Meisal determinations that when glucose, protein hydrolysate, sodium oleate and sodium acetate were given in this way, they did not prevent the decrease in lower fatty acids which normally accompanies fasting.

The influence of the lipid content of the blood plasma of the cow and particularly of the level of phospholipids on the secretion of milk fat has been studied at some length by Saarinen (214, 215). He found that there was no significant correlation between the level of phospholipids in the blood and the average fat percentage in the day's production of milk, and concluded (very wisely in view of much earlier work by others) that the correlations often obtained between the phospholipids of the blood serum and the percentage of fat in the milk result not from phospholipids being precursors of milk fat, but from the fact that high phospholipid levels in blood are correlated with

high levels of other lipid fractions. The results of a series of experiments on the importance of the fat content of the diet, which were carried out between 1928 and 1944, have been published by Loosli & Maynard (216). In seventeen experiments in which a low fat ration (2·7% fat) was compared with a high fat ration (6·5%) containing the same amount of digestible nutrients, the high fat ration was believed to give rise on the average to 4·1% more milk.

Since the previous review of this series was prepared (1) little further knowledge has accumulated regarding the precursors of milk protein. It remains probable, as indicated in that earlier review, that the protein in milk arises partly from blood amino-acids (187) and partly from the glycoproteins which occur in the globulin fraction of the blood (201).

The secretion of phosphorus in cows' milk has been studied with the aid of radioactive phophorus by Comar and his associates (217). The active element was fed as a disodium phosphate solution to Jersey cows in single doses. With three cows at the peak of lactation and giving 2–3 gal. of milk daily, 12–15% of the radioactive phosphorus was accounted for in the milk in the 10 days which followed the treatment. With three cows in mid-lactation giving 1½–2 gal. daily the corresponding figure was 7–8%. About 25% of the radioactive phosphorus of the milk was in the casein and the remainder in the acid serum phosphate. None was present in the butterfat. In more recent experiments by Kleiber, Smith & Ralston (190) the P^{32} compound was injected into two cows via the jugular vein. The amount of P^{32} in the milk reached a maximum of 1·2% of the injected dose per litre of milk 5 hr. after injection, and a week later P^{32} was still appearing in the milk. In a week 20 and 23% of the injected dose was secreted in the milk, an appreciable proportion of it being in the casein.

Work has continued in recent years on the enzymes of the mammary tissue. Thus Dempsey, Bunting & Wislocki (218) have shown the presence of an acid phosphatase which Folley suggests is the phosphomonoesterase A_2 in the phosphatase classification which he and Kay (219) published in 1936. Folley & Greenbaum (220) have studied the concentration of alkaline phosphatase in the rat mammary gland during pregnancy, lactation and involution. Half-way through the gestation period a very marked rise in concentration occurred, and the maximum, which was about 12 times higher than the value at the beginning of pregnancy, was reached almost immediately after parturition. It was maintained at that level until the young were weaned and then rapidly decreased. The phosphatase activity in the lactating gland of the rat was found by Folley & Greenbaum to be about one-third that of the kidney. The distribution of alkaline phosphatase among the mammary tissues has recently been studied in detail by Dempsey *et al.* (218) and the subject has been reviewed by Folley (186).

The enzyme of the mammary gland which has received most attention in the past few years is arginase. As mentioned in earlier reviews (1, 211) this enzyme had been shown to be present in bovine mammary tissue in 1938 by Shaw & Petersen (221), and in that of the mouse in 1941 by Greenstein, Jenrette, Mider & White (222). In more recent years Folley & Greenbaum showed its presence in the mammae of the rat (111), and later (186, 220) they found that it was present also in the mammary gland of the guinea-pig, rabbit and goat. When the amount of the enzyme present per gram of moist mammary tissue of the rat was taken as 100, the corresponding amount in the mouse was 35 or 61 depending on the stage of lactation, whereas the amounts for the guinea-pig, rabbit, goat and cow were much lower, ranging from 1 to 7, the values for the cow tissue being 2 and for the goat 3. The amount present in the mammary tissue of herbivorous animals was therefore much lower than in that of the others. In the mammary tissue of the pregnant rat the content of arginase increased relatively slightly until the 5th day of lactation, when it rose rapidly and reached a peak of about 12 times the original value and then declined rapidly at weaning (220). Folley & Greenbaum (221) suggest that the rapid increase in arginase content which begins in the mammary gland of the rat at about the 5th day of lactation may be necessary to enable the gland to meet part of the increasing demands of lactation by a greater formation of sugar from protein. Folley and his colleagues (112, 223, 225) have studied the effect of adrenalectomy on the arginase content of the liver, mammary gland and kidney in lactating rats. When the rats were adrenalectomized on the 4th day of lactation and autopsied on the 17th day, the

arginase levels per gram of moist tissue were significantly reduced. In the mammary gland the arginase level was reduced to about a seventh of the original value, as compared with reduction to about one-third in the liver and one-half in the kidney. In the normal lactating rat the arginase content per gram of moist liver tissue was found to be nine times the corresponding value for the mammary gland and nearly thirty times the value for the kidney. Folley & Greenbaum discuss the possibility that the reduction in milk yield which follows adrenalectomy may be caused by a reduction in the ability of the liver to prepare some of the milk precursors by gluconeogenesis from protein, and of the mammary gland to utilize protein by gluconeogenesis. Later, Folley & Greenbaum(226) studied the effect of experimental diabetes on tissue arginase levels in rats and concluded that decreases in the liver levels were secondary to changes in the rate of amino-acid katabolism, and that there is therefore not necessarily a direct relationship between adrenal cortex hormones and tissue arginase.

Folley & Greenbaum(227) found the optimum pH of the arginase from rat mammary and liver tissue to be 9·45. At this pH the enzyme was not inhibited by excessive substrate concentrations, but at lower pH inhibition progressively increased with increase in substrate concentration. For the enzyme to be active manganese must be present, and it has now been shown that cobalt cannot effectively replace manganese. The enzyme of the mammary gland appeared to be identical with that of the liver.

Of other enzymes which exist in the bovine mammary tissue Kelly(228) has published evidence for the presence of a diacetinase which differs from the tributyrinase of the gland. He found it possible to heat the dried tissue to a point at which the tributyrinase activity was destroyed but a portion of the original diacetinase activity remained. Kay(229) has shown that the tributyrinase in cows' milk is light-sensitive. Milk at below 5° C. exposed to bright sunshine in a layer 1 cm. deep lost 40% of its tributyrinase activity in 10 min. and 80% in 30 min. Red light had least effect and blue light most effect. The addition of riboflavin increased the rate of destruction.

Much work has been done in the past few years to determine whether dietary factors exist which are specifically required for normal lactation as distinct from normal growth. It will be of value to refer briefly to some of the more important investigations made under this heading. The general principle common to all the work in this field is to study growth, reproduction and lactation in rats or mice when fed highly purified diets and also when fed the same diets supplemented with different amounts of extracts likely to contain substances with vitamin-like activity. In 1947 Nelson & Evans(230, 231) published results of their long-term experiments carried out in this way with rats. One of their typical basal diets consisted of casein 24%, sucrose 62%, hydrogenated vegetable oil 8%, cod-liver oil 2% and salts 4%. The effect of high protein contents and high fat contents in the diet were also tested as well as the effect of many vitamins. They found that lactation was the function most adversely affected by inadequacies in the diet; growth was the next, and reproductive behaviour in the male the least affected. From the results they obtained in their first experiments Nelson & Evans were able to devise an improved basal diet containing eight crystalline vitamins of the B complex and an improved salt mixture. On this diet the requirements of reproduction and growth of rats which had been weaned appeared to be satisfied, but from the loss of weight of the lactating rats and suboptimal growth of the young during lactation it was concluded that lactation has additional dietary requirements. A higher protein level in the diet appeared to give slightly better lactation results, but high fat levels were deleterious.

In much of this type of work the investigations must continue till several generations of rats have been studied. Nelson & Evans(231), however, have described a short-term procedure of 3 weeks which they claim is of great value in investigations of this kind. It involves putting the adult animals on the experimental diets at parturition, and is claimed by the authors to give satisfactory agreement with results obtained by long-term work. One would imagine, however, that in most instances confirmation by long-term investigations would be essential. In later experiments the same workers(232) showed that the maternal leucocyte counts were low and

lactation performance poor on the basal diets, but that both were improved when synthetic pteroylglutamic acid was added. The improvement with liver eluate powder was, however, still more marked, so that while folic acid is probably essential for lactation, another factor or factors present in liver are also required. Folley, Henry & Kon (233) have described experiments extending over 5 years in which growth, reproduction and lactation were studied with rats receiving a diet of purified constituents and supplemented in various ways. They found that the addition of fresh liver to the basal diet gave almost normal performance, whereas the addition of a liver extract or small amounts of milk gave only partial improvement. Partial improvement was obtained also with a biotin extract but not with pure biotin or folic acid. Clearly some other factor or factors present in fresh liver are essential. The suggestion has been made (234) that alfalfa is a source of these factors.

Evidence that the 'animal protein factor', B_{12} is one of the factors concerned is now available. In 1948 Zucker & Zucker (235) showed that some dietary factor essential for normal lactation in rats, which they termed 'Zoopherin', was supplied by liver and also by *crude* casein but not by *pure* casein or brewers' yeast. They concluded that the factor was not a protein, but appeared to be associated with animal protein. In one part of the investigations at Wisconsin (236) it was found that rats receiving beef protein reared 80–90% of their young and those receiving casein about 67%, whereas when pork protein was fed the results were very variable and sometimes the rats reared less than 30% of their young. With the pork diets which led to poor lactation fresh liver restored the lactation to that obtained with beef protein and casein. In a later publication (237) a method was described for assaying vitamin B_{12}, and by this method it was found that the beef samples used in the experiments which have just been cited contained twice as much B_{12} as the pork sample which gave normal lactation, while the pork sample giving poor lactation contained only a mere trace of B_{12}. As the authors point out, it is possible that the ruminant is supplied with ample amounts of B_{12} through the micro-organisms of the rumen, whereas the pig is much more dependent on its diet for this substance.

The value of factors such as biotin, folic acid and xanthopterin for lactation in mice has been shown in recent years by Cerecedo & Mirone (238, 239) and Fenton & Cowgill (240). The work as a whole shows that many of the vitamins of the B complex are essential for lactation and that lactation cannot be normal without adequate amounts of B_{12}. Whether there are other dietary factors essential for lactation and whether any of these factors are specific for lactation as distinct from growth, and are not simply growth factors required in greater amount during lactation, has still to be established.

ANATOMICAL AND RELATED ASPECTS

Udder growth in the cow during the last 2 months of pregnancy and during lactation has been studied by Altman (241). He endeavoured to obtain the udder capacity as represented by the internal volume of the alveoli, ducts and cisterns. He concluded that during lactation new alveoli are not formed and existing alveoli do not increase in size. After making several assumptions he calculated that on the day of parturition the number of cells per section of alveolus was about nineteen as compared with thirteen in late pregnancy. The number per section altered significantly as lactation proceeded. Mitosis was rarely noted, the corresponding figure being one mitosis per 16,000 or 70,000 cells. There are several other conclusions of this type which will interest those who are studying the development and anatomy of the mammary gland. Matthews, Swett & Fohrman have made an extensive investigation of the weight and capacity of udders from dairy heifers of different ages (242) and from cows of different producing abilities and ages and at different stages of lactation (243). Udder weights in fifty heifers between 3 and 30 months of age increased on an average by 0·59 lb./month. The estimated standard weight was about 2·2 lb. at 6 months and 12·9 lb. at 24 months. The capacity of the udders from heifers ranging in age from 9 to 30 months increased by about 0·44 lb./month. In the work on cows data was collected for 473 udders. The increase in weight and capacity was found to be greatest between the first and second

lactation. After 6 years of age the increases were not significant. The average weights and capacities found for lactating and dry udders of Holsteins and Jerseys were as follows:

Breed	No. of cows	Average age (years)	Weight of udder (lb.)	Capacity of udder lb.)
		Lactating		
Holsteins	78	6	49·4	67·9
Jerseys	86	7	38·0	55·0
		Non-lactating		
Holsteins	69	5	27·0	37·4
Jerseys	66	$5\frac{1}{2}$	24·3	28·8

As lactation advanced variable but definite decreases occurred in udder weight and less definite decreases in capacity. Highly significant correlations were found between producing ability and udder weight in both lactating and dry cows.

What is likely to prove a valuable method of preserving a record of the structure of an excised mammary gland has been described by Bechtel & McLeod(244), who were particularly interested in the tubulo-alveolar structures. The excised gland is suspended in a wooden frame, milked out and then injected with a dilute solution of acetic acid. Some hours later the acid is drained off and neoprene latex (Du Pont) injected through each teat until the gland is filled. The udder is then immersed in commercial HCl for about 2 weeks to 'corrode' the tissue. The final product can be washed and dried or immersed in weak formalin for display purposes. Latex of different colours can be used for injection in the milk-containing structure, the veins and the arteries. Photographs of some latex 'corrosions' are given. Swett & Matthews(245) have also used latex in studying the circulatory system of the cow's udder. They have produced an excellently illustrated bulletin on the subject. A detailed study of the lymphatic and arterial systems in the bovine udder has been made by El Hagri(246, 247). The two papers together contain some fifteen anatomical diagrams and eight photographs and cannot be reviewed adequately here. An account of the lymph nodes is given, and the anatomical distribution and classification of the lymph vessels and their relation to each other are discussed. The arterial blood distribution was investigated in ten udders: five by dissection, four by X-rays and stereoscopic methods followed by dissection, and one by the celloidin injection corrosion method. For a valuable general review on structural features of the mammary tissue reference should be made to an article by Richardson(248).

Changes in the volume of the teats of the bovine mammary gland have been measured by Peeters, Massart & Oyaert(249) by means of plethysmography, and the morphology of the teat, particularly in relation to milking and trauma, has been investigated by Petersen, Knodt & Boyd(250). Petersen and his associates in dissecting 400 teats found that 90% of them showed pathological features, most of which were believed to be the result of milking. The position and extent of the annular fold which marks off the gland cistern from the teat cistern was found to vary considerably in different teats. In some teats there were two or three folds.

It has often been thought that highly developed external mammary veins were a sign of high milk-producing capacity, but in work carried out over a number of years in America(251) no correlation was found between the size of the external mammary veins and milk production in 106 Holstein cows and 89 Jerseys.

Findlay & Yang(252) have made a detailed histological study of the so-called 'sweat glands' in the bovine. This work forms part of a long-term investigation on the dissipation of heat by the skin of cattle. In their first publication, which is well illustrated, they discuss the structure, distribution and dimensions of the sweat glands in twenty-one different body regions of five 3–4-year-old Ayrshire cows, and they also give a few data for calves and embryos. They found that each hair follicle is accompanied by an arrector pili muscle, a 'sweat' gland and a sebaceous gland, a combination which they term a 'hair follicle unit'. The 'sweat' glands appeared to have a poor blood supply and to be apocrine, so that it is unlikely that the 'sweat' glands of the Ayrshire

cow function as effectively in heat regulation as the sweat glands of man. A study of the sweat glands of tropical cattle is also being made.

At intervals during the last 20 years a number of investigators, particularly in Russia, have claimed that there is a high correlation between the number of 'sweat' glands per unit area in the skin of the ears of cattle and their milk yields(253, 254, 255, 256, 257, 258). Zamjatina(258) claimed that for 283 Simmental cows the correlation coefficient between the number of sweat glands per sq.mm. in the upper corner of the left ear and the milk yield was 0·741. The average number of glands per sq.mm. was 8·58, but in thirty-seven animals with low milk yield the corresponding figure was only 3·01. Burcev(253) used 1336 Red German cattle for a somewhat similar study. He also obtained a high correlation between the sweat-gland count in the ear and the milk yield, and he claimed that there was no difference in the number of sweat glands per unit area of skin between the right and left ears or between different regions of the same ear, and that the number did not depend on age or sex, but was characteristic of the particular animal being studied. If these claims were true, a useful estimate of the milk-producing capacity of a calf or cow could be made simply by counting the sweat glands in a piece of skin such as might be obtained from the ear by a branding punch. Findlay, Goodall & Yang(259) have therefore investigated the matter in Ayrshires. Contrary to the claims of Burcev for Red German cattle, they found that the number of sweat glands per unit area of skin varied very greatly indeed from one part of the ear to another, and even from one field to another in any particular part. They also found that the number per unit area in the ear of the calf is much greater than for the corresponding area in the ear of the cow. They found no significant correlation between the sweat-gland count in the ear and the milk yield, and it seems certain from their results that the sweat-gland count could not be used as an indication of milk-producing capacity in Ayrshires.

In 1934 Swett & Matthews(260) described a method of selecting dairy calves on the basis of mammary development. The method depended on the fact that the extent of mammary development when the calves were 3–4 months old was directly related to the heifer's later productive ability. Much more recently Schabinger & Knodt(261) have published the results of their investigations on the relationship between mammary development and body weight of Holstein and Guernsey calves at 3 and 6 months old. They found highly significant correlation coefficients between mammary development, as measured by length and width of udder quarters, and body weight when the calves were 3 months old but not when they were 6 months old. For ages varying from 8 days to 3 months the correlations were also significant, but not for ages ranging from 8 days to 6 months.

In 1941 Gaines(262) claimed that within a dairy breed and within a herd the yield of fat-corrected milk produced by a cow is proportional to the 1·07 power of its live weight, where the live weight is determined in the first month of lactation, and he concluded that it would be sound practice to express lactation performance in terms of milk-energy yield for the first 8 months of lactation divided by the live weight in the first month of lactation, FCM_8/W_1. This claim has been disputed by Kleiber & Mead(263) on the grounds that Gaines's data and conclusion were statistically unreliable, that age corrections may be hidden corrections for body weight, since cows continue to grow after their first lactation, and that size differences within a herd are so small. They state that an independent estimate of the effect of body weight can be accomplished by using the theory that productive capacity is proportional to metabolic size, i.e. to the 0·75 power of the body weight. They suggest that a valuable criterion of selection for breeding dairy cattle would be the 'relative lactation capacity' which they define as the milk energy per day (the mean of 10 months' production) divided by the mean metabolic size (in kg. $W^{0·75}$). A year later Gaines(264) produced further evidence in support of his original claim and stated that the weight of experimental evidence distinctly supported the formula involving body weight rather than the one put forward by Kleiber & Mead involving metabolic body size, and in a still more recent paper Gaines and his colleagues(265) again maintain the importance of using body weight in obtaining a formula to express what Brody terms 'lactational drive'.

The causes and extent of variations in the weight of cows have been investigated by Allen(266,

267), who draws attention to the fact that in much experimental work involving the determination of live weights there is a notable lack of uniformity in the weighing methods used by different workers, and frequently no details of the method used are given. In Allen's investigations dairy cattle were kept in stalls mounted on scales for periods of 10 days and weighed at half-hourly intervals without disturbing their normal routine. The animals showed great uniformity in the times at which maximum, minimum and mean weights for the day were reached. The lowest weight occurred just before feeding, and this was the least variable figure. For milking cows its variability was reduced by deducting the weight of the milk at the subsequent milking. The maximum occurred about 2½ hr. after the evening feed. It appeared that the mean weight for the day could be obtained fairly accurately by a single weighing made 6–7 hr. after the morning feed.

Work on conformation and its relation to milk yield continues in different parts of the world. Leighton & Graves(268) have endeavoured to determine what relationship exists between the inclination of the rump and the inclination of the udder, production ability and breeding efficiency in cows. From 500 photographs of 155 Holstein-Friesians taken under standard conditions once a year, the slopes of the rump and udder were determined. There was no significant correlation between either of the slopes and producing ability, nor between the slope of the rump and the number of services per conception. A study of the data for the daughters of the different sires suggested that the slope of the rump and of the udder were inherited characters, and that they were inherited independently of each other. For the goat van Leeuwen(269) claimed that there was a positive correlation between general conformation and milk yield at 1, 2 and 3 years old, but animals with well-formed udders did not produce more milk on the average than those with udders which appeared to be less well-formed.

A new instrument for standardizing methods of taking measurements of cattle has been described by Olbrycht(270), who points out that body measurements are made in so many different ways that it is difficult to compare zoometric description of animals of different breeds made by different workers. He proposes a standard method to get over this difficulty. The accuracy of linear body measurements of dairy cattle has also been discussed by Touchberry & Lush(271).

Brody and his colleagues have continued their series of publications on growth and development in cattle. In a bulletin(272) on the growth and metabolism of beef cattle they conclude that, like surface area, metabolism increases with approximately the 0·6 power of the body weight rather than with the 0·67 or 0·73 power as previously supposed(273). The ventilation rate, on the other hand, appears to be directly proportional to the body weight. In a later bulletin(274) Brody and his co-workers describe their experiments on the heat production and cardiorespiratory activities of Jersey cattle during gestation and lactation. Data were obtained on the influence of gestation and lactation on the resting heat production, on body weight, and on the pulse rate, respiration rate and pulmonary ventilation rate of sixteen Jersey heifers, and these data were compared with corresponding data on heat production and body weight of rats. Some of the average results for cows are summarized below:

	In dry cows when served at 18 months old	At the peak of lactation
Total heat production (kg.cal./day)	8,000	13,000
Pulse rate (beats/min.)	60	80
Pulmonary ventilation (l./min.)	60	125
Respiration rate (per min.)*	25	45
Tidal air (l.)	2	4

* Varies with environmental temperature.

With dry animals there tended to be a small decrease in heat production during the first 3 months or so of pregnancy, but this was followed by a gradual increase as gestation continued and by a very marked increase during lactation. For example, in one Jersey cow which gave about 4½ gal. of milk at the peak of lactation, the heat production per sq.m. of body surface per day remained at approximately 2000 kg.cal. from the age of 6 months to half-way through the gesta-

tion period at about 25 months. Towards the end of gestation, the heat production was still below 2800 kg.cal., but during the lactation which followed, it reached a peak of almost 4000 kg.cal.

Blaxter(275) and Blaxter & Price(276, 277) have made a detailed study of the normal variation which occurs in the heart rate, respiration rate and body temperature of dairy cows. It was found that standing increased the heart rate by 5–7 beats/min. as compared with lying, but that rumination had only a very small effect and increased the rate by only 1–3 beats/min. The ingestion of food had a most marked effect, with 4 lb. concentrates there was an increase at the maximum of 11 beats/min., and with 20 lb. kale the increase was 16 beats/min. After a feed consisting of 12 lb. hay and 20 lb. kale the rate accelerated from 54 to a maximum of 90 beats/min. There appeared to be no change in heart rate, respiration rate and body temperature during the first 6 months of pregnancy, but in the last 3 months the heart rate increased by 15–40% and the rectal temperature by about 1° F. The respiratory rate increased only very slightly in the latter part of pregnancy. It was, however, very sensitive to environment, and increased by nearly 100% for every 10° C. rise in temperature. Over short periods milk yield was positively and significantly correlated with the resting heart rate, and when yields for longer periods were corrected for the normal decline in lactation, deviations from the normal decline were also highly correlated with heart rate. In discussing their results Blaxter & Price present evidence which suggests that the solids-not-fat percentage in milk is determined at least in part by the total metabolism of the cow, which in turn is related to the plane of nutrition.

BLOOD

Changes in the level of vitamin A and carotene in blood plasma associated with parturition and lactation in dairy cows have been studied by Sutton *et al.*(278, 279, 280). In the later work (280) the breeds used were Ayrshire, Guernsey, Jersey and Holstein—seven cows of each breed. The cows were winter calvers kept indoors and fed a diet consisting of good-quality alfalfa hay, maize silage and concentrates. Blood samples were analysed at weekly intervals beginning 4 weeks before and 2 weeks after the expected parturition date. Additional samples were taken within 24 hr. before and after parturition and also 3 days after. In all the breeds vitamin A and carotene levels decreased fairly steadily during the last 3 weeks of pregnancy and more rapidly in the week following parturition to values about 50% less than those at the first test; thereafter they increased again. Carotene intake was not measured, so that it is not known what proportion of these changes was caused by physiological changes associated with the reproductive cycle, but average total outputs of carotene and vitamin A in the colostrum during the first 3 days after calving approximated to 50,000 μg., and this would account at least in part for the large decrease which occurred in the blood levels immediately after parturition.

Statistical analysis of 773 determinations of blood calcium and phosphorus and 613 observations for haemoglobin, erythrocyte count, leucocyte count and differential leucocyte percentages was made for Jersey and Holstein cows by Rusoff & Piercy(281). Poor-quality hay did not appear to influence the level of calcium or the content of cellular consituents, but the phosphorus and haemoglobin values appeared to be affected in the experimental herd getting the poorer ration. Variations in the blood cell counts of healthy cattle have also been studied by Ferguson, Irwin & Beach(282).

Papers have appeared recently on the carbon dioxide content of bovine blood(283) and on the blood types in pairs of identical bovine twins(284). Schultz & Smith(285) have shown that the ketone content of the blood of goats is markedly increased when butyric, caproic, caprylic and capric acids are administered orally, but not when acetic acid is given.

SALIVA

A large number of saliva samples from dairy cattle have been studied by Reid & Huffman(286). The average water content was 99·1% and the pH was 8·53. The pH did not vary appreciably

when taken at 6 hr. intervals over a 24 hr. period. The average ascorbic acid content was 0·15 mg/.100 ml., while the corresponding figure for the plasma was 0·47 mg./100 ml.

MISCELLANEOUS FACTORS AFFECTING MILK SECRETION

A comprehensive review of the literature on the effects of temperature, humidity, air movement and solar radiation on the behaviour and physiology of cattle and other farm animals has been published this year by Findlay (287). Most of the first eight chapters of the bulletin deal with the effects of tropical environments on the respiratory function, body temperature and heart rate of cattle and on the composition of the blood and milk of both European and tropical breeds. The function of the skin of cattle in the dissipation and absorption of heat is also discussed. The remaining seven chapters are concerned mainly with modern methods of measuring body and skin temperatures, respiratory function, heart rate and other physiological functions. Since most of the relevant literature of the past 20 years has been reviewed in this bulletin by Findlay, it will suffice here if attention is confined to some of the most recent work on the effect of climate on dairy cattle.

Riek & Lee in Australia have carried out an extensive series of experiments on the effect of exposing Jersey cows (288) and calves (289) for 7 hr. twice a week to atmospheres having dry-bulb temperatures ranging from 85 to 110° F. and absolute humidities from 6 to 16 grains of moisture per cu.ft. of dry air, the air moving at the rate of 60 ft./min. The cows were milked immediately before and after exposure. The various items studied included rectal temperature, pulse rate, respiratory rate and volume, evaporative loss, the composition of the blood and milk, and the behaviour of the animals. The rectal temperatures of the cows rose from 101° F. to as high as 107° F. at the high dry-bulb temperatures, and the respiration rate from 25 to 160 per min. The calves were found to have a lower thermal tolerance than the cows. Continuing their earlier work on heat tolerance (290, 291) Seath & Miller in Louisiana have now made heat-tolerance comparison between Jerseys and Holsteins (292) under conditions in which the atmospheric temperature on the days of the experiment ranged from 65 to 93° F. The body temperatures of the Holsteins averaged 0·7 or 0·8° F. higher than those of the Jerseys under the same conditions, and in general the Holsteins were less heat-tolerant. On hot days they had a much greater tendency than Jerseys to wallow in water and mud. Changes in air temperature appeared to have a much greater effect than changes in humidity, and Holsteins with a large percentage of white area on their body surface did not appear to differ from other Holsteins in their heat tolerance. The same authors have investigated the effects of shade and of sprinkling the cows with water in the presence of both still and moving air on the body temperature and on the respiration and pulse rates of Jersey cows which had been exposed to sunshine and high atmospheric temperatures (293, 294, 295). All these three physiological functions were lowered by shade alone, but sprinkling followed by shade was very much more effective. When air was used in addition to sprinkling, the body temperature, respiration rate and pulse rate rapidly fell to what were regarded as normal values. Further experiments are still required to determine to what extent cows can lose heat by sensible and insensible perspiration. In further work Seath (296) has discussed in some detail the heritability of heat tolerance in Holstein and Jersey cattle. In a comparison between Friesians and Zebus in Kenya it has been shown by Kendall (297) that in the cattle native to the tropics the rise in body temperature associated with a rise in atmospheric temperature is much less than in high-grade Kenya Friesians. Gaalaas (298, 299) has also made some important observations on the general question of heat tolerance in cows. He calculated what he terms the heat-tolerance coefficient from the body temperature, and concluded that the stage of lactation and gestation had little effect on this coefficient.

The effect of climate on the composition of milk has been studied by Heinemann (300), who found that in bulked milk in Missouri there was an average increase of 0·067% in the total solids content of separated milk and 0·093% in the fat content of whole milk for each 10° F. decrease in atmospheric temperature. Overman (301) at Illinois also found that in cows of six different

breeds fat percentage was highest in winter. Changes in the protein, ash and non-fatty solids tended to follow those of the fat, but lactose percentage tended to change slightly in an inverse direction to fat percentage. Rees(302), on the other hand, in Tasmania, found that fat percentages tended to drop in the winter and that the solids-not-fat percentages were greatest in spring and autumn. These differences between findings in such widely separated areas as the United States and Tasmania are doubtless caused by nutritional differences, such as the state of the pasture, rather than by the direct action of climate itself on the physiology of the cows. The influence of the season of the year on the composition of milk in Pretoria, particularly with regard to rainfall and temperature, has been discussed by Bakalor(303), and the influence of many factors, such as the season of the year, age and stage of lactation, general manangement and the conformation and structure of the udder, by van Rensburg(304) in Onderstepoort.

The relative merits of keeping cows loose in pens as compared with having them tied in 'stanchion barns' have been investigated by Graves, Dawson & Kopland(305). Groups of seven cows running loose in 'pen barns', one group being fed a very adequate concentrate ration and one on a more limited ration, produced 19 and 7% more milk than two similar groups kept tied in 'stanchion barns'. The general condition of the animals in the 'pen barns' was also better.

Dice(306) has continued his earlier work on the effect of turning the cows out of the byre during the day in extremely cold weather, which was cited in the previous review(1), and which showed that provided the animals are sheltered from wind and snow and have dry bedding and adequate rations, their condition and milk production is not adversely affected by exposure to extremely cold temperatures. With heifers housed in open and closed sheds from November to March, Dice now finds that the heifers in the closed sheds showed slightly greater gains in weight and slightly greater skeletal growth than those in open sheds. The latter consumed more protein and total nutrients for the same gain in weight.

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REFERENCES

(1) Smith (1945). *J. Dairy Res.* **14**, 195.
(2) MacGregor (1941). *Vet. J.* **97**, 340.
(3) Bartlett (1949). *J. Dairy Sci.* **32**, 36.
(4) Asdell (1929). *J. Dairy Sci.* **32**, 60.
(5) Asdell (1949). *J. Dairy Sci.* **32**, 45.
(6) Gilmore (1949). *J. Dairy Sci.* **32**, 71.
(7) Sykes (1949). *J. Dairy Sci.* **32**, 92.
(8) Laing (1945). *Vet. Rec.* **57**, 275.
(9) Hancock (1948). *Vet. Rec.* **60**, 513.
(10) Burg, van den. *Tijdschr. Diergeneesk.* **73**, 810.
(11) Korkman (1947). *Suom. Maataloust. Seur. Julk.* **64**, no. 1.
(12) Hornaday (1947). *Vet. Med.* **42**, 129.
(13) Wille (1947). *Mh. Vet. Med.* **2**, 17.
(14) Durrell (1947). *Canad. J. comp. Med.* **11**, 290.
(15) Folley, Greenbaum & Roy (1948). *J. Endocrinol.* **6**, 121.
(16) Greenbaum (1947). *Brit. Goat Soc. Yearb.* p. 47.
(17) Hancock (1947). *Vet. Rec.* **59**, 639.
(18) Quinlan, Roux, Aswegen & Lange (1948). *Onderstepoort J. vet. Sci.* **23**, 269.
(19) Mercier & Salisbury (1947). *J. Dairy Sci.* **30**, 747.
(20) Mercier & Salisbury (1947). *J. Dairy Sci.* **30**, 817.
(21) Laing (1945). *J. agric. Sci.* **35**, 25.
(22) Trimberger & Davis (1945). *J. Dairy Sci.* **28**, 659.
(23) Homb (1947). *Meld. Norg. LandbrHøgsk.* **27**, 315.
(24) Eri & Homb (1947). *Meld. Norg. LandbrHøgsk.* **27**, 351.

(25) Berge (1945). *Norsk Landbr.* **11**, 284.
(26) Pegg (1948). *Qd agric. J.* **66**, 169.
(27) Woodward (1945). *J. Dairy Sci.* **28**, 209.
(28) Frick, Mann & Johnson (1947). *J. Dairy Sci.* **30**, 631.
(29) Phillips (1947). Mimeograph, Univ. Coll., Aberystwyth, Wales.
(30) Phillips & Davies (1949). *J. Dairy Res.* **16**, 1.
(31) Phillips, Davies & Brown (1949). *J. Dairy Res.* **16**, 129.
(32) *Home Fmr*, **14**, 16 (1947).
(33) Phillips, Brown & Davies (1949). *J. Dairy Res.* **16**, 283.
(34) Lonka (1946). *Maataloust. Aikakausk.* **18**, 147.
(35) Krizenecky & Kudlicka (1944). *Z. Tierz. ZüchtBiol.* **56**, 299.
(36) Livesay & Bee (1945). *J. anim. Sci.* **4**, 13.
(37) Veiga, Paiva & Chieffi (1947). *Bol. Industr. anim.* N.S. **9**, 32.
(38) Weaver, Wettstein & Horwood (1947). *Quart. Bull. Mich. agric. Exp. Sta.* **30**, 180.
(39) Briquet & Abreu (1948). *Rev. Agric.* (Piracicaba, S. Paulo), **23**, 219.
(40) Casida, Barrett & Lloyd (1946). *J. Dairy Sci.* **29**, 553.
(41) Brigatti (1948). *Zootec. e Vet.* **3**, 418.
(42) Hamilton & Laing (1946). *J. Anat., Lond.*, **80**, 194.
(43) Rowson & Dowling (1949). *Vet. Rec.* **61**, 191.
(44) Asdell, Alba & Roberts (1945). *J. Anim. Sci.* **4**, 277.
(45) Laing (1945). *J. agric. Sci.* **35**, 1.
(46) Laing (1945). *J. agric. Sci.* **35**, 72.
(47) Phillips & Spitzer (1946). *J. Dairy Sci.* **29**, 407.
(48) Bayley, Cobbs & Barrett (1950). *J. Dairy Sci.* **33**, 24.
(49) Salisbury (1946). *J. Dairy Sci.* **29**, 695.
(50) Cheng, Casida & Barnett (1949). *J. Anim. Sci.* **8**, 81.
(51) Willett (1950). *J. Dairy Sci.* **33**, 43.
(52) Bratton, Foote, Musgrave & VanDemark (1949). *J. Dairy Sci.* **32**, 604.
(53) Hurst & LaMaster (1948). *J. Anim. Sci.* **7**, 541.
(54) Swanson (1948). *J. Dairy Sci.* **31**, 680.
(55) Swanson (1949). *J. Dairy Sci.* **32**, 345.
(56) Knodt & Salisbury (1946). *J. Dairy Sci.* **29**, 285.
(57) Salisbury & Knodt (1947). *J. Dairy Sci.* **30**, 361.
(58) Salisbury & Bratton (1948). *J. Dairy Sci.* **31**, 817.
(59) Foote & Bratton (1949). *J. Dairy Sci.* **32**, 856.
(60) Ghosh, Casida & Lardy (1949). *J. Anim. Sci.* **8**, 265.
(61) VanDemark, Salisbury & Bratton (1949). *J. Dairy Sci.* **32**, 353.
(62) Johnston & Mixner (1948). *J. Anim. Sci.* **7**, 440.
(63) Mixner & Johnston (1949). *J. Dairy Sci.* **32**, 570.
(64) Johnston, Stone & Mixner (1949). *J. Dairy Sci.* **32**, 574.
(65) Sallman & Birkeland (1948). *Amer. J. Physiol.* **152**, 271.
(66) Salisbury, Knodt & Bratton (1948). *J. Anim. Sci.* **7**, 283.
(67) Almquist, Prince & Reid (1949). *J. Dairy Sci.* **32**, 543.
(68) Prince, Almquist & Reid (1949). *J. Dairy Sci.* **32**, 849.
(69) Branton, Bratton & Salisbury (1949). *J. Dairy Sci.* **32**, 292.
(70) Bretschneider (1948). *Vlaam. diergeneesk. Tijdschr.* **17**, 8. *Anim. Breed. Abstr.* **16**, 320.
(71) Bretschneider & Iterson (1947). *Proc. Acad. Sci. Amst.* **50**, 88. *Anim. Breed. Abstr.* **16**, 320.
(72) Bretschneider (1947). *Tijdschr. Diergeneesk*, **62**, 640. *Anim. Breed. Abstr.* **16**, 124.
(73) Greeson & Zlontnik (1948). *Quart. J. micr. Sci.* **89**, 219.
(74) Folley (1947). *Brit. med. Bull.* **5**, 130.
(75) Folley (1947). *Brit. med. Bull.* **5**, 135.
(76) Reece & Leathem (1945). *Proc. Soc. exp. Biol., N.Y.*, **59**, 122.
(77) Trentin & Turner (1947). *J. Anim. Sci.* **6**, 501.
(78) Cowie & Folley (1944). *Nature, Lond.*, **154**, 302.
(79) Cowie & Folley (1947). *Endocrinology*, **40**, 274.

(80) Folley & Malpress (1944). *J. Endocrinol.* **4**, 1.
(81) Folley, Malpress & Young (1945). *J. Endocrinol.* **4**, 181.
(82) Day & Hammond (1945). *J. agric. Sci.* **35**, 150.
(83) Spriggs (1945). *Vet. Rec.* **57**, 519.
(84) Mixner & Turner (1943). *Res. Bull. Mo. agric. Exp. Sta.* no. 378.
(85) Malpress (1947). *Brit. med. Bull.* **5**, 161.
(86) Van Heuverswyn, Folley & Gardner (1939). *Proc. Soc. exp. Biol., N.Y.*, **41**, 389.
(87) Cowie (1948). *J. Endocrinol.* **6**, 145.
(88) Trentin & Turner (1947). *Endocrinology*, **41**, 127.
(89) Petersen, Knodt, Ludwick & Pomeroy (1944). *Proc. Soc. exp. Biol., N.Y.*, **57**, 332.
(90) Mixner (1947). *J. Dairy Sci.* **30**, 578.
(91) Leonard & Reece (1941). *Endocrinology*, **28**, 65.
(92) Smithcors & Leonard (1942). *Endocrinology*, **31**, 454.
(93) Smithcors (1945). *Proc. Soc. exp. Biol., N.Y.*, **59**, 197.
(94) Folley & Young (1945). *J. Endocrinol.* **4**, 194.
(95) Fawns, Folley & Young (1945). *J. Endocrinol.* **4**, 205.
(96) Folley, Malpress & Young (1945). *J. Endocrinol.* **4**, 212.
(97) Cotes, Crichton, Folley & Young (1949). *Nature, Lond.*, **164**, 992.
(98) Young (1947). *Brit. med. Bull.* **5**, 155.
(99) Robinson (1947). *Lancet*, **253** (6464), 90.
(100) Robinson (1947). *Brit. med. Bull.* **5**, 164.
(101) Meites & Turner (1947). *Proc. Soc. exp. Biol., N.Y.*, **64**, 488.
(102) Meites & Turner (1947). *Amer. J. Physiol.* **150**, 394.
(103) Liu & Turner (1949). *J. Dairy Sci.* **32**, 881.
(104) Li & Kalman (1946). *J. Amer. chem. Soc.* **68**, 285.
(105) Li & Fraenkel-Conrat (1947). *J. biol. Chem.* **167**, 495.
(106) White (1946). *Physiol. Rev.* **26**, 574–608.
(107) Nelson, Gaunt & Schweizer (1943). *Endocrinology*, **33**, 325.
(108) Cowie & Folley (1947). *J. Endocrinol.* **5**, 14.
(109) Cowie & Folley (1947). *J. Endocrinol.* **5**, 24.
(110) Folley & Greenbaum (1948). *J. Endocrinol.* **5**, 236.
(111) Folley & Greenbaum (1946). *Biochem. J.* **40**, 46.
(112) Folley & Greenbaum (1948). *Biochem. J.* **43**, 581.
(113) Cowie & Folley (1947). *J. Endocrinol.* **5**, 9.
(114) Hammond (1948). 'Festskrift til Professor Per Tuff, 70 ar', p. 134. Oslo: Grøndahl and Sons, Boktrykkeri.
(115) Malpress (1947). *Brit. med. Bull.* **5**, 161.
(116) Folley, Malpress & Young (1945). *J. Endocrinol.* **4**, 181.
(117) Hohlweg, W. (1947). *Milchwissenschaft*, **2**, 169.
(118) Narayan & Bhalerao (1947). *Curr. Sci.* **16**, 29.
(119) Holland & Campbell (1946). *Proc. Ann. Conf. N.Z. Soc. Anim. Prod.* p. 104.
(120) Spriggs (1945). *Vet. Rec.* **57**, 519.
(121) Day & Hammond (Jr.) (1945). *J. agric. Sci.* **35**, 150.
(122) Nottbohm (1948). Reprinted from *Molkereiztg (Hildesh.)* (24).
(123) Marshall, Becker & Arnold (1946). *J. Dairy Sci.* **29**, 524.
(124) Dyrendahl (1946). *Skand. VetTidskr.* **36**, 478; 513 (B).
(125) Meites & Turner (1947). *J. Anim. Sci.* **6**, 499.
(126) Peeters & Massart (1947). *J. Endocrinol.* **5**, 166.
(127) Peeters, Vandeplassche & Massart (1946). *Vet. Rec.* **58**, 627.
(128) Bartlett, Folley, Rowland, Curnow & Simpson (1948). *Nature, Lond.*, **162**, 845.
(129) Graham (1934). *J. Nutrit.* **7**, 407.
(130) Graham (1934). *Biochem. J.* **28**, 1368.
(131) Folley & White (1936). *Proc. roy. Soc.* B, **120**, 346.
(132) Ludwig & Mutzenbacher (1939). *Hoppe-Seyl. Z.* **258**, 195.
(133) Pitt-Rivers & Randall (1945). *J. Endocrinol.* **4**, 221.

(134) Deanesly & Parkes (1945). *J. Endocrinol.* **4**, 356.
(135) Blaxter (1945). *J. Endocrinol.* **4**, 237.
(136) Blaxter (1945). *J. Endocrinol.* **4**, 266.
(137) Blaxter (1946). *J. agric. Sci.* **36**, 117.
(138) Blaxter, Reineke, Crampton & Petersen (1949). *J. Anim. Sci.* **8**, 307.
(139) Van Landingham, Hyatt & Weakley (1946). *J. Dairy Sci.* **29**, 533.
(140) Van Landingham, Hyatt, Weakley & Henderson (1947). *J. Dairy Sci.* **30**, 576.
(141) Seath, Branton & Groth (1945). *J. Dairy Sci.* **28**, 509.
(142) Reece (1946). *J. Dairy Sci.* **29**, 533.
(143) Reece (1947). *J. Dairy Sci.* **30**, 313.
(144) Reece (1947). *J. Dairy Sci.* **30**, 574.
(145) Reece (1950). *J. Dairy Sci.* **33**, 126.
(146) Booth, Elvehjem & Hart (1947). *J. Dairy Sci.* **30**, 443.
(147) Allen, Dow, Logan & MacKenzie (1948). *Sci. Agric.* **28**, 340.
(148) McQuillan, Trikojus, Cambell & Turner (1948). *Brit. J. exp. Path.* **29**, 93.
(149) Swanson & Knodt (1949). *J. Dairy Sci.* **32**, 257.
(150) Thomas, Moore & Sykes (1949). *J. Dairy Sci.* **32**, 278.
(151) Thorbek, Hansen & Moustgaard (1948). *J. Anim. Sci.* **7**, 291.
(152) Owen (1948). *Biochem. J.* **43**, 235.
(153) Owen (1948). *Biochem. J.* **43**, 243.
(154) Blaxter (1948). *J. agric. Sci.* **38**, 1.
(155) Blaxter (1948). *J. agric. Sci.* **38**, 20.
(156) Blaxter (1948). *J. agric. Sci.* **38**, 207.
(157) Archibald (1945). *J. Dairy Sci.* **28**, 941.
(158) Kemmerer, Bolomey, Vavich & Davies (1946). *Proc. Soc. exp. Biol., N.Y.*, **63**, 309.
(159) Hibbs & Krauss (1947). *J. Anim. Sci.* **6**, 161.
(160) Van Landingham, Henderson & Weakley (1944). *J. Dairy Sci.* **27**, 385.
(161) Robertson (1945). *J. Endocrinol.* **4**, 300.
(162) Bruger & Silberbush (1946). *J. clin. Endocrinol.* **6**, 565.
(163) Hurst & Turner (1948). *Res. Bull. Mo. agric. Exp. Sta.* no. 417.
(164) Moore & Sykes (1948). *Yearb. U.S. Dep. Agric.* 1943–7 (Science in Farming), p. 107.
(165) Dyrendahl (1949). Bulletin published from the Royal Veterinary College, Stockholm.
(166) Robinson (1947). *Lancet*, **253** (6472), 385.
(167) Borrow, Clayton & Hems (1949). *J. chem. Soc.* S 185.
(168) Chalmers, Dickson, Elks & Hems (1949). *J. chem. Soc.* 3424.
(169) Bailey, Bartlett & Folley (1949). *Nature, Lond.*, **163**, 800.
(170) Ely & Petersen (1941). *J. Dairy Sci.* **24**, 211.
(171) Knodt, Reid, Williams & Kesler (1947). *J. Dairy Sci.* **30**, 580.
(172) Knodt, Reid, Williams & Kesler (1949). *J. Dairy Sci.* **32**, 637.
(173) Dodd & Foot (1947). *J. Dairy Res.* **15**, 1.
(174) Miller & Petersen (1941). *J. Dairy Sci.* **24**, 225.
(175) Dodd & Foot (1949). *J. Dairy Res.* **16**, 14.
(176) Dodd, Foot & Henriques (1949). *J. Dairy Res.* **16**, 301.
(177) Ward & Smith (1949). *J. Dairy Sci.* **32**, 17.
(178) Korkman (1948). *K. LantbrAkad. Tidskr.* **87**, 161.
(179) Smith (1947). *J. Dairy Sci.* **30**, 703.
(180) Petersen & Gilmore (1946). *J. Anim. Sci.* **5**, 399.
(181) Newton & Newton (1948). *J. Pediat.* **33**, 698.
(182) Smith & Petersen (1945). *J. Dairy Sci.* **28**, 431.
(183) Smith & Petersen (1945). *J. Dairy Sci.* **29**, 45.
(184) Whittleston (1946). *N.Z. Dairy Exptr*, **21**, 8.
(185) Folley (1947). *Brit. med. Bull.* **5**, 142.
(186) Folley (1949). *Biol. Rev.* **24**, 316.
(187) Shaw (1946). *J. Dairy Sci.* **29**, 183.
(188) Popják & Beeckmans (1949*a*). *Biochem. J.* **44**, xxxvii.

(189) Popják & Beeckmans (1949*b*). *Biochem. J.* **44**, xxxvi.
(190) Kleiber, Smith & Ralston (1948). *Proc. Soc. exp. Biol., N.Y.*, **69**, 354.
(191) Trucco, Caputto, Leloir & Mittelman (1948). *Arch. Biochem.* **18**, 137.
(192) Grant (1935). *Biochem. J.* **29**, 1905.
(193) Grant (1936). *Biochem. J.* **30**, 2027.
(194) Malpress & Morrison (1950). *Biochem. J.* **46**, 307.
(195) Knodt & Petersen (1945). *J. Dairy Sci.* **28**, 415.
(196) Knodt & Petersen (1946). *J. Dairy Sci.* **29**, 121.
(197) Folley & French (1949). *Biochem. J.* **45**, 117.
(198) Friedmann (1949). *Biochem. J.* **44**, 117.
(199) Hammond (1913). *Quart. J. exp. Physiol.* **6**, 311.
(200) Smith, Howat & Ray (1938). *J. Dairy Res.* **9**, 310.
(201) Reineke, Williamson & Turner (1941). *J. biol. Chem.* **138**, 83.
(202) Knodt & Petersen (1946). *J. Dairy Sci.* **29**, 115.
(203) Petersen, Shaw & Visscher (1941). *J. Dairy Sci.* **24**, 139.
(204) Folley & French (1949). *Biochem. J.* **45**, 270.
(205) Folley & French (1948). *Nature, Lond.*, **161**, 933.
(206) Folley & French (1948). *Biochem. J.* **43**, lv.
(207) Folley & French (1949). *Biochem. J.* **44**, xlv.
(208) Folley & French (1949). *Nature, Lond.*, **163**, 174.
(209) Popják, Folley & French (1949). *Arch. Biochem.* **23**, 509.
(210) Popják, French & Folley (1950). *Biochem. J.* **46**, xxviii.
(211) Smith (1941). *J. Dairy Res.* **12**, 78.
(212) Phillipson (1947–1948). *Nutrit. Abstr. Rev.* **17**, 12.
(213) Mann & Shaw (1947). *J. Dairy Sci.* **30**, 183.
(214) Saarinen (1944). *Suom. Maataloust. Seur. Julk.* **57**, 1.
(215) Saarinen (1945). *Suom. Maataloust. Seur. Julk.* **60**, 1.
(216) Loosli & Maynard (1945). *Fm Res.* (N.Y. St. and Cornell agric. Exp. Sta.), **11**, 3.
(217) Comar, Becker, Arnold, Krienke & Davis (1947). *J. Dairy Sci.* **30**, 557.
(218) Dempsey, Bunting & Wislocki (1947). *Amer. J. Anat.* **81**, 309.
(219) Folley & Kay (1936). *Ergebn. Enzymforsch.* **5**, 159.
(220) Folley & Greenbaum (1947). *Biochem. J.* **41**, 261.
(221) Shaw & Petersen (1938). *Proc. Soc. exp. Biol., N.Y.*, **38**, 631.
(222) Greenstein, Jenrette, Mider & White (1941). *J. Nat. Cancer Inst.* **1**, 687.
(223) Folley & Greenbaum (1947). *Nature, Lond.*, **160**, 364.
(225) Cowie, Folley, French & Greenbaum (1949). *J. Endocrinol.* **6**, ii.
(226) Folley & Greenbaum (1948). *J. Endocrinol.* **6**, x.
(227) Folley & Greenbaum (1948). *Biochem. J.* **43**, 537.
(228) Kelly (1945). *J. Dairy Sci.* **28**, 799.
(229) Kay (1946). *Nature, Lond.*, **157**, 511.
(230) Nelson & Evans (1947). *Arch. Biochem.* **12**, 213.
(231) Nelson & Evans (1947). *Arch. Biochem.* **12**, 229.
(232) Nelson & Evans (1947). *Arch. Biochem.* **13**, 265.
(233) Folley, Henry & Kon (1947). *Brit. J. Nutrit.* **1**, 39.
(234) Bowland, Ensminger & Cunha (1948). *Arch. Biochem.* **16**, 257.
(235) Zucker & Zucker (1948). *Arch. Biochem.* **16**, 115.
(236) Henderson, Schweizert, Mozingo & Elvehjem (1948). *J. Nutrit.* **36**, 479.
(237) Register, Lewis, Thompson & Elvehjem (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 167.
(238) Cerecedo & Mirone (1947). *Arch. Biochem.* **12**, 154.
(239) Mirone & Cerecedo (1947). *Arch. Biochem.* **15**, 324.
(240) Fenton & Cowgill (1947). *J. Nutrit.* **33**, 703.
(241) Altman (1945). *Vestn. Zhivotnov.* **1**, 85.
(242) Matthews, Swett & Fohrman (1949). *Tech. Bull. U.S. Dep. Agric.* no. 993.
(243) Matthews, Swett & Fohrman (1949). *Tech. Bull. U.S. Dep. Agric.* no. 989.
(244) Bechtel & McLeod (1945). *Amer. J. vet. Res.* **6**, 17.

(245) Swett & Matthews (1949). *Tech. Bull. U.S. Dep. Agric.* no. 982.
(246) El Hagri (1945). *Vet. J.* **101**, 27 and 51.
(247) El Hagri (1945). *Vet. J.* **101**, 75.
(248) Richardson (1947). *Brit. med. Bull.* **5**, 123.
(249) Peeters, Massart & Oyaert (1948). *Experientia*, **4**, 36.
(250) Petersen, Knodt & Boyd (1944). *J. Dairy Sci.* **27**, 646.
(251) U.S. Dep. Agric. *Rep. Admr agric. Res.* 1943–4, pp. 106–7 (Washington, D.C.).
(252) Findlay & Yang (1950). *J. Anat., Lond.* (in the Press).
(253) Burcev (1937). *Usp. zooteh. Nauk.* **4**, 137.
(254) Konjkov (1930). *Izv. Kazan S.H. Inst.* (cited by Burcev (253)).
(255) Podoba (1934). Cited by Burcev (253).
(256) Stakalic (1945). *Izv. Akad. Nauk S.S.S.R.* (Ser. biol), pp. 722, 729.
(257) Podoba (1933). Cited by Stakalic (256).
(258) Zamjatina (1946). *Trud. novosibirsk. seljskohoz. Inst.* no. 7, 135.
(259) Findlay, Goodall & Yang (1950). *J. Dairy Res.* (in the Press).
(260) Swett & Matthews (1934). *Yearbook of Agriculture*, p. 175. U.S. Dep. Agric.
(261) Schabinger & Knodt (1948). *J. Dairy Sci.* **31**, 95.
(262) Gaines (1941). *J. Dairy Sci.* **24**, 795.
(263) Kleiber & Mead (1945). *J. Dairy Sci.* **28**, 49.
(264) Gaines (1946). *J. Dairy Sci.* **29**, 259.
(265) Gaines, Davis & Morgan (1947). *J. Dairy Sci.* **30**, 273.
(266) Allen (1946). *Bull. Vt agric. Exp. Sta.* no. 534.
(267) Allen (1946). *J. Dairy Sci.* **29**, 544.
(268) Leighton & Graves (1947). *J. Dairy Sci.* **30**, 25.
(269) Leeuwen van (1948). *Maandbl. LandbVoorlDienst.* **5**, 36.
(270) Olbrycht (1945). *Emp. J. exp. Agric.* **13**, 199.
(271) Touchberry & Lush (1950). *J. Dairy Sci.* **33**, 72.
(272) Brody, Romfort, Kibler & Worstell (1947). *Res. Bull. Mo. agric. Exp. Sta.* no. 404.
(273) Brody (1945). *Bioenergetics and Growth.* New York: Reinhold.
(274) Brody, Worstell, Ragsdale & Kibler (1948). *Res. Bull. Mo. agric. Exp. Sta.* no. 412.
(275) Blaxter (1943). *Vet. J.* **99**, 2.
(276) Blaxter & Price (1945). *Vet. J.* **101**, 18.
(277) Blaxter & Price (1945). *Vet. J.* **101**, 39.
(278) Sutton & Soldner (1943). *J. Dairy Sci.* **26**, 740.
(279) Sutton & Soldner (1945). *J. Dairy Sci.* **28**, 859.
(280) Sutton, Kaeser & Soldner (1945). *J. Dairy Sci.* **28**, 933.
(281) Rusoff & Piercy (1946). *J. Dairy Sci.* **29**, 831.
(282) Ferguson, Irwin & Beach (1945). *J. infect. Dis.* **76**, 24.
(283) Dennis & Harbaugh (1946). *Amer. J. vet. Res.* **7**, 37.
(284) Owen (1945). *Science*, **102**, 400.
(285) Schultz & Smith (1949). *J. Dairy Sci.* **32**, 817.
(286) Reid & Huffman (1949). *J. Dairy Sci.* **32**, 123.
(287) Findlay (1950). *Bull. Hannah Dairy Inst.* no. 9.
(288) Riek & Lee (1948). *J. Dairy Res.* **15**, 219.
(289) Riek & Lee (1948). *J. Dairy Res.* **15**, 227.
(290) Seath & Miller (1946). *J. Dairy Sci.* **29**, 199.
(291) Seath & Miller (1946). *J. Dairy Sci.* **29**, 465.
(292) Seath & Miller (1947). *J. Anim. Sci.* **6**, 24.
(293) Seath & Miller (1947). *J. Dairy Sci.* **30**, 255.
(294) Seath & Miller (1948). *J. Dairy Sci.* **31**, 361.
(295) Seath & Miller (1948). *J. Anim. Sci.* **7**, 251.
(296) Seath (1947). *J. Dairy Sci.* **30**, 137.
(297) Kendall (1948). *Vet. J.* **104**, 112.
(298) Gaalaas (1945). *J. Dairy Sci.* **28**, 555.
(299) Gaalaas (1947). *J. Dairy Sci.* **30**, 79.

(300) Heinemann (1947). *J. Dairy Sci.* **30**, 757.
(301) Overman (1945). *J. Dairy Sci.* **28**, 305.
(302) Rees (1947). *Aust. J. Dairy Tech.* **2**, 3.
(303) Bakalor (1948). *Fmg S. Afr.* **23**, 271.
(304) Rensburg (1946). *Fmg S. Afr.* **21**, 217.
(305) Graves, Dawson & Kopland (1947). *Circ. U.S. Dep. Agric.* no. 763.
(306) Dice (1947). *Bull. N. Dak. agric. Exp. Sta.* no. 344.

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